



IntechOpen

IntechOpen Series
Oncology, Volume 9

Cancer Immunotherapy

Cellular Mechanisms, Therapeutic Advances
and Emerging Frontiers

Edited by Thamiris Becker Scheffel



Cancer Immunotherapy - Cellular Mechanisms, Therapeutic Advances and Emerging Frontiers

Edited by Thamiris Becker Scheffel

Published in London, United Kingdom

Contributors

Abolaji Samson Olagunju, Anca-Florina Zgura, Anna Bilotta, Apostolos Gogakos, Areti Katsouda, Asma Ahsan, Ayodeji Mathias Adegoke, Ayomide Oluwadarasimi Adebesein, Chang Liu, Deborah Dardano, Eleni Litsou, Festus Adebayo Atiba, Francesco Bignoli, Gaber El-Saber Batiha, Giordana Di Mauro, Giuliana Ciappina, Giuseppe Donato, Ioannis Passos, Jawad Akhtar, John Oluwafemi Teibo, Karine Rech Begnini, Khaled Barakat, Laya K. Sathyan, Lisa M. James, Li Zhang, Mariacarmela Santarpia, Maria Mironidou-Tzouveleki, Marwan Al-Hajeili, Massimiliano Berretta, Myrtw Tzinevi, Patrizia Carroccio, Piyush Kumar, Rodica-Maricela Anghel, Saba Ismail, Saira Dar, Srinivas Naidu, Stefania Chipuc, Thamaris Becker Scheffel, Thomas Kanteres, Thomas Rallis, Titilade Kehinde Ayandeyi Teibo, Vasileios Theocharidis, Yi-Jun Tu

© The Editor(s) and the Author(s) 2025

The rights of the editor(s) and the author(s) have been asserted in accordance with the Copyright, Designs and Patents Act 1988. All rights to the book as a whole are reserved by INTECHOPEN LIMITED. The book as a whole (compilation) cannot be reproduced, distributed or used for commercial or non-commercial purposes without INTECHOPEN LIMITED's written permission. Enquiries concerning the use of the book should be directed to INTECHOPEN LIMITED rights and permissions department (permissions@intechopen.com)

Violations are liable to prosecution under the governing Copyright Law.



Individual chapters of this publication are distributed under the terms of the Creative Commons Attribution 4.0 License which permits commercial use, distribution and reproduction of the individual chapters, provided the original author(s) and source publication are appropriately acknowledged. If so indicated, certain images may not be included under the Creative Commons license. In such cases users will need to obtain permission from the license holder to reproduce the material. More details and guidelines concerning content reuse and adaptation can be found at <http://www.intechopen.com/copyright-policy.html>.

Notice

Statements and opinions expressed in the chapters are those of the individual contributors and not necessarily those of the editors or publisher. No responsibility is accepted for the accuracy of information contained in the published chapters. The publisher assumes no responsibility for any damage or injury to persons or property arising out of the use of any materials, instructions, methods or ideas contained in the book.

First published in London, United Kingdom, 2025 by IntechOpen

IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 167-169 Great Portland Street, London, W1W 5PF, United Kingdom

For EU product safety concerns: IN TECH d.o.o., Prolaz Marije Krucifikse Kozulić 3, 51000 Rijeka, Croatia, info@intechopen.com or visit our website at intechopen.com.

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

Cancer Immunotherapy – Cellular Mechanisms, Therapeutic Advances and Emerging Frontiers

Edited by Thamaris Becker Scheffel

p. cm.

This title is part of the Oncology Book Series, Volume 9

Series Editor: Thomas J. FitzGerald

Topic: Cancer Diagnostics and Treatment

Topic Editor: Michael Gibson

Print ISBN 978-0-85466-326-2

Online ISBN 978-0-85466-325-5

eBook (PDF) ISBN 978-0-85466-327-9

ISSN 3049-8864

If disposing of this product, please recycle the paper responsibly.

IntechOpen Book Series

Oncology

Volume 9

Aims and Scope of the Series

The field of oncology has undergone extraordinary change and progress over the past several decades. Today, information is evolving at a rapid rate, with standards of management far different than standards of care applied during the training of most practicing oncologists. Oncology practitioners in all disciplines must remain current to optimize patient care. Basic and translational science remain critical in developing process improvements for patient care. The closer we understand the mechanism, the more we can improve targeted therapies and apply them to patient care. The pace of information is moving faster than at any previous time in history, and all oncology disciplines must remain current to provide excellent service to patients. The modern oncologist must be fluent in using big data and the volume of information generated in clinical trials. As we move closer to personalized patient care based on genomics and molecular biomarkers, the modern oncologist has to be nimble in assessing all available information and how this would be applied to each patient, balanced by the clinical status and medical co-morbidities of each patient. Targeted therapies can bring new and different sequelae, and oncology teams need to remain fluent in managing the consequences of therapy and primary management. In this book series, we will present how modern care has progressed in multiple disease areas and how modern oncology teams need to adapt in order to manage the cancer patients of today successfully. Surgery, radiation therapy, and medical oncology are practiced today with the support of exceptional modern technology, and in this series, we will review how these improvements are applied to each disease site to maintain excellence in patient care.

Meet the Series Editor



Dr. FitzGerald is the professor and chair of the Department of Radiation Oncology at UMass Chan Medical School in the USA. He serves as one of the principal investigators of the Imaging and Radiation Oncology Core (IROC) service for the National Cancer Institute clinical trials program and is directly involved in the quality assurance of clinical trials in the National Clinical Trials Network (NCTN). Dr. Fitzgerald manages NCTN clinical trials with a real-time pre-therapy review of imaging and radiation therapy treatment objects to ensure the care plan complies with study objectives and the patient stage has been assigned to the correct study. His basic science interest is in hematopoietic stem cell biology and cellular adhesion molecules as they pertain to therapeutic resistance and mitigation of injury from therapy.

Meet the Volume Editor



Thamiris Becker Scheffel is a pharmacist with a residency in Clinical Pharmacy and Clinical Analysis and holds a Ph.D. in Cellular and Molecular Biology. She is currently the Coordinator of the Innovation and Development of Radiopharmaceuticals team (NIDERA) and a Specialist in Research and Development at the Brain Institute (InsCer) of PUCRS, Brazil, where she leads projects in radiopharmacy applied to oncology and neurodegenerative diseases. Her academic journey includes a doctoral thesis on brain tumors, the purinergic system, and immune checkpoint blockade, followed by a postdoctoral fellowship at the Cellular Signaling and Plasticity Laboratory at UFRGS, focused on tumor heterogeneity. Passionate about translating scientific discoveries into practical health solutions, she is dedicated to advancing innovation in the detection and treatment of complex diseases.

Contents

Preface	XIII
Section 1	
Building the Foundations: Global Contexts, Molecular Keys, and Clinical Insights	1
Chapter 1	3
Cancer Immunotherapy in Sub-Saharan Africa: Current Landscape, Barriers and Strategic Directions <i>by John Oluwafemi Teibo, Festus Adebayo Atiba, Ayomide Oluwadarasimi Adebesein, Abolaji Samson Olagunju, Titilade Kehinde Ayandeyi Teibo, Ayodeji Mathias Adegoke, Marwan Al-Hajeili and Gaber El-Saber Batiha</i>	
Chapter 2	19
HLA-Based Immunotherapy for Cancer <i>by Lisa M. James</i>	
Chapter 3	39
Predictive Biomarkers of Immunotherapy <i>by Laya K. Sathyan, Srinivas Naidu and Piyush Kumar</i>	
Section 2	
Navigating the Tumor Landscape: Immune Evasion and Interactions	51
Chapter 4	53
The Science of PD-1/PD-L1 Pathway in Gliomas: Far beyond the Obvious <i>by Thamiris Becker Scheffel and Karine Rech Begnini</i>	
Chapter 5	81
The Tumor Microenvironment: Immunological Interactions and Therapeutic Targeting <i>by Anca-Florina Zgura, Rodica-Maricela Anghel and Stefania Chipuc</i>	
Chapter 6	103
Immune Cells and Their Role in Immunosuppressive Tumor Microenvironment of Head and Neck Cancer <i>by Eleni Litsou</i>	

Chapter 7	143
Role of Crosstalk between Microbiota and Immune System in Cancer Immunotherapy	
<i>by Giuliana Ciappina, Patrizia Carroccio, Francesco Bignoli, Mariacarmela Santarpia, Giordana Di Mauro and Massimiliano Berretta</i>	
Section 3	
Immunotherapy in Action: Strategies for Different Cancers	155
Chapter 8	157
Immunology and Gliomas: Present and Future Opportunities in Diagnostics and Therapy	
<i>by Anna Bilotta, Deborah Dardano and Giuseppe Donato</i>	
Chapter 9	177
Immunotherapy in the Treatment of Melanoma	
<i>by Areti Katsouda, Thomas Rallis, Apostolos Gogakos, Vasileios Theocharidis, Thomas Kanteres, Myrtw Tzinevi, Ioannis Passos and Maria Mironidou-Tzouveleki</i>	
Chapter 10	201
Liver Cancer Immune Microenvironment and Immunotherapy	
<i>by Chang Liu, Yi-Jun Tu and Li Zhang</i>	
Section 4	
Shaping the Future: mRNA Vaccines and Oncolytic Therapies	223
Chapter 11	225
Neoantigen mRNA Cancer Vaccines: How Deep Learning Is Advancing Design and Breaking Barriers	
<i>by Saba Ismail and Khaled Barakat</i>	
Chapter 12	271
Oncolytic Virus Therapy: Advancements and Applications in Cancer Care	
<i>by Asma Ahsan, Saira Dar and Jawad Akhtar</i>	

Preface

Cancer immunotherapy has transformed medicine, revealing cancer as a complex interplay between tumors, the immune system, and the surrounding environment. This book presents a curated collection of chapters that cover both foundational concepts and practical applications, guiding readers through the current landscape of cancer immunotherapy.

Section 1 – *Building the Foundations: Global Contexts, Molecular Keys, and Clinical Insights* introduces an overview of cancer immunotherapy. Chapter 1, *Cancer Immunotherapy in Sub-Saharan Africa: Current Landscape, Barriers, and Strategic Directions*, examines challenges and opportunities in diverse healthcare settings. Chapter 2, *HLA-Based Immunotherapy for Cancer*, explains how HLA molecules guide personalized treatments. Chapter 3, *Predictive Biomarkers of Immunotherapy*, demonstrates how biomarkers aid in improving treatment decisions.

Section 2 – *Navigating the Tumor Landscape: Immune Evasion and Interactions* explores how tumors interact with the immune system. Chapter 4, *The Science of PD-1/PD-L1 Pathway in Gliomas: Far Beyond the Obvious*, examines how gliomas evade immune detection. Chapter 5, *The Tumor Microenvironment: Immunological Interactions and Therapeutic Targeting*, describes the cells and signals that influence tumor growth and treatment response. Chapter 6, *Immune Cells and Their Role in the Immunosuppressive Tumor Microenvironment of Head and Neck Cancer*, focuses on the roles of immune cells in head and neck cancers. Chapter 7, *Role of Crosstalk between Microbiota and Immune System in Cancer Immunotherapy*, highlights how the microbiome and systemic factors shape therapy outcomes.

Section 3 – *Immunotherapy in Action: Strategies for Different Cancers* presents applications across tumor types. Chapter 8, *Immunology and Gliomas: Present and Future Opportunities in Diagnostics and Therapy*, reviews current and emerging glioma therapies. Chapter 9, *Immunotherapy in the Treatment of Melanoma*, covers advances in melanoma treatment and the role of biomarkers. Chapter 10, *Liver Cancer Immune Microenvironment and Immunotherapy*, highlights immune checkpoints in liver cancer and strategies to target them.

Section 4 – *Shaping the Future: mRNA Vaccines and Oncolytic Therapies* looks at emerging strategies. Chapter 11, *Neoantigen mRNA Cancer Vaccines: How Deep Learning is Advancing Design and Breaking Barriers*, presents mRNA vaccines and computational tools that improve their design.

Chapter 12, *Oncolytic Virus Therapy: Advancements and Applications in Cancer Care*, describes viruses that selectively kill cancer cells and enhance immune responses.

Gratitude is expressed to all the authors for their contributions, sharing knowledge and insights that made this book possible. Thanks are also extended to the editorial

team and assistants, whose dedication ensured smooth organization and refinement of the collection.

It is hoped that this book serves as a practical and inspiring guide for researchers, clinicians, and students, offering a clear view of current knowledge and future directions in cancer immunotherapy. Each chapter represents a step in the journey to better understand and harness the immune system in the fight against cancer.

Thamiris Becker Scheffel

Brain Institute,
Pontifical Catholic University of Rio Grande do Sul,
Porto Alegre, Brazil

Section 1

Building the Foundations: Global Contexts, Molecular Keys, and Clinical Insights

Cancer Immunotherapy in Sub-Saharan Africa: Current Landscape, Barriers and Strategic Directions

John Oluwafemi Teibo, Festus Adebayo Atiba, Ayomide Oluwadarasimi Adebessin, Abolaji Samson Olagunju, Titilade Kehinde Ayandeyi Teibo, Ayodeji Mathias Adegoke, Marwan Al-Hajeili and Gaber El-Saber Batiha

Abstract

Cancer is one of the leading causes of death worldwide and a major global public health concern, especially in sub-Saharan Africa (SSA). Recent advances in the five pillars of cancer treatment: surgery, radiation, chemotherapy, targeted therapies, and immunotherapy have enhanced the treatment of cancers, with immunotherapy leading as a frontier in the treatment of cancers. However, in SSA, there are limitations to the application of this frontier treatment due to minimal or no representation of Africans in most clinical trials involving cancer immunotherapy. To achieve desired treatment successes in SSA, special attention needs to be paid to how these advances can be applied in SSA settings. In this chapter, we examine major barriers to the adoption of immunotherapy, such as high treatment costs, a lack of biomarker testing and genomic sequencing, underrepresentation in clinical trials, and insufficient healthcare infrastructure. We concluded with strategic proposals for overcoming these constraints, including greater funding for cancer research in SSA, extension of local clinical trials, capacity training, and policy reforms. Addressing these disparities is critical for ensuring equitable access to immunotherapy and improving cancer outcomes throughout SSA.

Keywords: cancer immunotherapy, sub-Saharan Africa, cancer burden, oncology access, healthcare equity, access disparities

1. Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide [1]. According to the World Health Organisation (WHO), in 2020, cancer accounted for 1 in every 6 deaths, that is, about 10 million people, with breast and lung cancer

causing about 40% of all such deaths (WHO, 2025) [2, 3]. This high rate of morbidity and mortality has necessitated continual advancements in treatment procedures. However, prevention of cancer remains an important part, as approximately 30 to 50% of cancers can currently be prevented by avoiding risk factors and through early detection [3].

Over the years, cancer therapy has evolved from traditional and conventional approaches like surgery, chemotherapy, and radiotherapy to more sophisticated, targeted strategies like nanomedicine to precision-targeted therapy [4, 5]. However, cancer immunotherapy remains one of the most advanced treatments for cancer worldwide, but it is not yet widely adopted in sub-Saharan Africa (SSA). This is particularly concerning because most approved immunotherapies are indicated for advanced-stage cancers, which is precisely the stage at which most patients in SSA are diagnosed, due to late presentation and diagnosis. However, patients from SSA are largely underrepresented in major cancer immunotherapy clinical trials. This lack of representation is rooted in the scarcity of genomic data specific to SSA populations, which is essential to capture the unique genetic characteristics of these patients [6, 7].

In this chapter, we present an overview of common traditional cancer treatments currently available. We further explore cancer immunotherapy in SSA in detail, critically examining the factors contributing to its limited implementation and proposing strategies to help the region advance and effectively integrate immunotherapy into cancer care in SSA.

2. Common traditional cancer treatments

2.1 Surgery

Surgical intervention remains the primary approach for localised cancers like breast cancer. Its aim is to remove malignant tissue entirely, although access is limited in low-resource settings, affecting around 25% of patients globally [8, 9].

2.2 Chemotherapy

Chemotherapy uses cytotoxic drugs to eliminate rapidly dividing cells. While effective, its non-selective nature often results in side effects such as immunosuppression, nausea, and infertility [10, 11]. Newer targeted delivery methods aim to reduce toxicity.

2.3 Targeted therapy

Targeted therapies selectively act on cancer cells with minimal impact on normal tissues. These include monoclonal antibodies (mAbs), polypeptides, and antibody-drug conjugates, offering high efficacy and lower toxicity [12, 13].

2.4 Radiotherapy

Radiotherapy applies ionising radiation to destroy cancer cells' DNA. Techniques like Intensity-mediated radiation therapy (IMRT) enhance precision and minimise harm to surrounding tissues [14].

2.5 Nanotherapy

Nanoparticles (1–1000 nm) are employed as smart drug carriers to improve cancer drug targeting and efficacy while reducing side effects [15].

2.6 Gene therapy

Gene therapy introduces or edits genetic material (e.g., CRISPR-Cas9) to correct mutations linked to cancer. Though promising, it remains largely experimental [16].

2.7 Photodynamic therapy (PDT)

PDT utilises light-activated compounds to generate reactive oxygen species that target and destroy cancer cells. It is non-invasive and tissue-selective, though some risk to healthy cells remains [17].

2.8 Proton therapy and boron neutron capture therapy (BNCT)

Proton therapy provides precision radiation with fewer side effects. BNCT uses boron-10 and neutron beams to selectively kill cancer cells [18].

2.9 Electrochemotherapy and theranostics

Electrochemotherapy enhances drug uptake *via* electric pulses. Theranostics combines diagnosis and therapy using radiolabelled compounds [19, 20].

2.10 Combinatorial ablation and immunotherapy

Ablation techniques paired with immunotherapy amplify immune responses, showing promise for metastatic cancers like hepatocellular carcinoma [21].

3. Major cancer treatment approaches

Table 1 gives an overview of cancer treatment approaches and an explanation of treatment components and mechanisms of action. These cancer treatment approaches

Cancer treatment approaches	Definition
Gene Therapy	The use of genes to fight cancer cells <i>via</i> apoptosis or suppression of cancer cells or silencing of certain genes.
Immunotherapy	Activation or suppression of body's immune system to fight cancer cells.
Radiotherapy	Use of a radioactive source to obliterate cancer cells.
Laser Therapy	Use of laser beams at very narrow and extremely focused wavelength to destroy neoplastic cells in a target area.
Targeted Therapy	Targeted drugs can be roughly classified into two categories: small molecules and macromolecules (e.g., mAbs, polypeptides, antibody-drug conjugates, and nucleic acids)

Cancer treatment approaches	Definition
Photodynamic Therapy (PDT)	PDT is treatment based on light-sensitive compounds activated by specific wavelengths to destroy cancer cells.
Radiomics	Radiomics is quantification of tumour properties obtained from the analysis of medical images to treat cancer.
Pathomics	Pathomics relies on generation and characterisation of high-resolution tissue images to treat cancer.
Theranostics	This form of cancer therapy utilises simultaneous imaging and therapy to fight cancer.
Nanoparticles	Introduction of nanoparticles (1–1000 nm in size) to fight tumour cells within the body.
Extracellular Vesicles (EVs)	Extracellular vesicles are used for drug delivery to fight cancer cells, for example, exosomes (30–150 nm) and shed microvesicles (sMV) about 50–1300 nm [22].
Hormone/Endocrine Therapy	The use of hormones to treat cancer, for example, oestrogen receptor modulators for breast cancer treatment.
Thermal Ablation	Using heat <i>via</i> long exposure between 41 and 51°C to destroy cancer tissues.
Magnetic hyperthermia	This technique exploits superparamagnetic or ferromagnetic nanoparticles that can generate heat after stimulation with an alternating magnetic field [4].

Table 1.
Overview of cancer treatment approaches.

are treatment strategies that need to be utilised also in SSA to fully combat cancer within the region, to ensure adequate treatment and survival of cancer patients.

4. Cancer immunotherapy

Cancer immunotherapy is an innovative treatment that enhances the immune system and suppresses tumour cell growth and metastasis, and has shown prolonged therapeutic responses across several cancer types [23, 24]. Immunotherapy often consists of several forms: immune checkpoint inhibition, oncolytic virus therapy, cancer vaccination, adoptive cellular immunotherapy (ACT), dendritic cell therapy, cytokine treatment, and antibody-drug conjugate (ADC), which have shown promising results in treating various diseases. Immunotherapy can be used as a monotherapy or combined with other treatment options, including chemotherapy for more effectiveness [25].

Immunotherapy has been demonstrated to be successful in the treatment of advanced tumours, including lung, prostate, and urothelial carcinoma [26, 27]. The CheckMate 227 study revealed the long-term effectiveness of nivolumab and ipilimumab in advanced non-small cell lung cancer [28]. The new KEYNOTE-B61 study confirmed Lenvatinib and pembrolizumab's anticancer effect in advanced non-clear-cell renal cell carcinoma, thereby promoting their usage as first-line treatments [29]. These results may contribute significantly to reducing cancer burden and mortality in SSA, where the majority of cancer patients come with late-stage cancers, limiting therapy choices and efficacy [30]. While efforts targeted at avoiding late-stage disease

appearance are necessary, effective therapies for advanced cancer are also required to lessen the region's high cancer death rate.

5. Advances in cancer immunotherapy

5.1 Monoclonal antibodies (mAbs)

Monoclonal antibodies (mAbs) are artificial proteins used as immunotherapies to cure and control some tumours [31]. There are currently four types of mAbs produced: human, chimeric, humanised, and murine – most of which are utilised in cancer immunotherapy. mAbs are the predominant form of immunotherapy utilised in SSA, specifically aimed at targeting distinct cancer markers. In recent years, mAb immunotherapy has been successfully used in clinical settings to treat and manage a variety of malignancies [32]. Today, scientists have created mAbs that block the signals that cause tumour development and invasion and target certain antigens in cancer cells.

5.2 Immune checkpoint blockade (ICB)

ICB represents a novel monoclonal antibody category that targets T-cell regulatory pathways to amplify antitumor immune responses [33]. It has shown increased survival rates for several cancers and the fight against tumours [34]. ICB enhances the longevity of anticancer responses by augmenting the immune system's antitumor activity and is intended to rejuvenate antitumor immune responses by disrupting coinhibitory signalling pathways and facilitating immune-mediated eradication of malignant cells [35, 36]. The primary targets for immune checkpoint blockade (ICB) are programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte-associated protein 4 (CTLA-4), and programmed cell death ligand 1 (PD-L1) [37]. Humanised monoclonal blocking antibodies directed against PD-L1 (atezolizumab, avelumab, and durvalumab), PD-1 (nivolumab, pembrolizumab, and cemiplimab), or CTLA-4 (ipilimumab) have been used in over 50 types of cancer, with the greatest efficacy shown in melanoma [38]. Interestingly, ipilimumab, an anti-CTLA-4 monoclonal antibody, was found to improve overall survival (OS) and progression-free survival (PFS) in phase II and phase III clinical studies of advanced melanoma patients who had previously received treatment [39], and it was the first immune checkpoint inhibitor approved by the US Food and Drug Administration (FDA) for this condition in 2011. Nonetheless, only a minority of patients have complete responses since their efficiency depends on optimal antitumor T-cell functionality and tumour mutational load [40].

5.3 Cytokine therapy

Cytokines serve as key regulators of both innate and adaptive immunity, facilitating communication among immune cells over short distances. Cytokine therapy for immune system activation in cancer patients represents a significant treatment approach and remains a crucial element in contemporary clinical cancer research. Interferon alpha (IFN α) and Peginterferon alpha 2b are authorised as adjuvant therapies for individuals with fully resected stage III or IV high-risk melanoma, and serve as first-line treatment for patients with metastatic renal cell carcinoma (mRCC),

specifically alfa-2a and alfa-2b in conjunction with bevacizumab. They are used for other various refractory malignancies. High-dose interleukin-2 (HDIL-2) is approved for the treatment of metastatic renal cell carcinoma and melanoma; however, its usage has declined due to the emergence of newer therapeutic agents [41, 42].

5.4 Adoptive cell therapies (ACT)

ACT includes using autologous immune cells, primarily T cells harvested from patients, growing and genetically altering them, and then infusing them into the patients to fight cancer or infections. ACT has gained attention due to the success of chimeric antigen receptor (CAR) T-cell therapy in treating blood cancer [43, 44]. CAR T-cell therapy, one of the primary ACT methods, has been successful in the treatment of haematological tumours [43]. Other known treatment approaches include TCR-engineered T-cell (TCR-T) therapy and tumour-infiltrating lymphocyte (TIL) therapy [45]. To improve the efficacy of ACT in solid tumours, gene modification strategies and the engineering of innate killer cells or macrophages to express TCR-like CARs, NK cell receptors, CARs, or transgenic TCRs have been employed [46].

5.5 Chimeric antigen receptor (CAR)T-cell therapy

Chimeric antigen receptor (CAR) T-cell therapy involves genetically modifying a patient's T cells to recognise and attack cancer cells [47]. Initially successful in haematological malignancies, solid tumours have not previously benefited from CAR T cells owing to low lymphocyte infiltration, tumour heterogeneity, and an immunosuppressive tumour microenvironment. However, recent studies have shown its efficacy in solid tumours by targeting the microenvironment [48, 49]. A ground-breaking trial reported that patients with advanced gastric or gastro-oesophageal junction cancers treated with CAR T-cell therapy experienced a 40% increase in overall survival compared to standard treatments [50]. As of 2024, the FDA has approved seven CAR T-cell therapies (ISCT, 2024) [51].

5.6 Oncolytic virus (OV) therapy

Oncolytic viruses (OV) are viruses that can be engineered to trigger selective cell death in cancer [52]. OVs have a simple mechanism of action: replication in cancer cells to cause lysis and eventual immunogenic cell death. Some OVs, including T-VEC, which is based on oncolytic herpes simplex virus type 1 (oHSV-1), have been approved for clinical use by the FDA, with others under preclinical evaluation [53]. In 2015, the FDA approved T-VEC for the treatment of metastatic melanoma [54]. Additionally, DELYTACT (oHSV-1 with G47Δ) was approved in 2021 for the treatment of glioblastoma in Japan [55]. T-VEC has shown remarkable therapeutic benefits for advanced melanoma patients and is presently approved for unresectable metastatic melanoma treatment [56].

6. Status of SSA in cancer immunotherapy advances

Cancer prevalence is increasing in SSA, and it is often defined by early age onset, aggressive illness, late-stage diagnosis, and poor survival [57]. SSA requires

immediate intervention to address the escalating crisis of cancer incidence and mortality. Data estimates indicate that, without prompt interventions, cancer mortality is projected to rise significantly to approximately 1 million deaths annually by 2030. The cancer challenge in SSA is significant and is expected to deteriorate swiftly without appropriate interventions.

The fight against cancer in SSA is more urgent now than ever. Deaths due to cancer in Africa are disproportionately greater than the corresponding mortality burden in other regions, with 756,600 deaths and 1,180,000 incidences in 2022 (Bray et al., 2024) [1, 58]. According to the International Agency for Research on Cancer (IARC), 693,487 Africans were estimated to die from cancer in 2018, with a projected rise to 1,429,812 deaths by 2040 – a staggering 106% increase in death rates between 2018 and 2040 [1, 59]. However, the incidence of cancer in SSA has doubled over the last 30 years, and without doubt, the numbers would double again in 20 years [60], resulting in a doubling of cancer mortality across Africa [61].

The increasing cancer burden in SSA can be linked to poor attention to cancer treatment within several healthcare systems in the region [61].

Immunotherapy has revolutionised cancer care worldwide and holds great promise for SSA, particularly due to its efficacy in treating advanced-stage cancers, which represent the majority of cases in the region. However, there is no robust documentation of the utilisation of immunotherapy in SSA [62].

In a study by Olatunji et al. [63], there were 292 respondents from 28 SSA nations. Of all responders, 46% said their clinics presently use cancer immunotherapy, and 29% said it is easily accessible. Out of the clinics that administered immunotherapy (n = 133), 44% evaluated the expression of the PD-L1 biomarker before administering immunotherapy, while 12% used genomic sequencing to evaluate the tumour mutational load biomarker. Among all respondents, 46% were aware of immunotherapy. 11% said they had received sufficient training to administer it. Of them (n = 33), 52% reported receiving training on how to handle immune-related side effects associated with administering immunotherapy. It was concluded that immunotherapy training and use are minimal and inadequate for the growing cancer burden.

Therefore, cancer immunotherapy in SSA faces systemic challenges that hinder its adoption and effectiveness, despite its potential to address advanced-stage cancers prevalent in the region.

Key barriers summarised in **Figure 1** include:

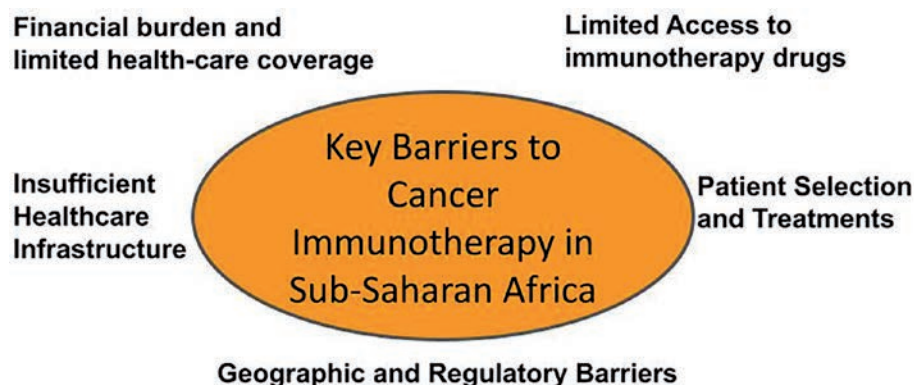


Figure 1.
 Key barriers to cancer immunotherapy in sub-Saharan Africa.

1. *Limited Access to immunotherapy drugs:* Despite the increasing rise in cancer across SSA, studies reveal that immunotherapy use is limited in SSA clinics, and even fewer, having adequate resources, trained staff, or easy access to immunotherapy drugs and diagnostic tools, largely due to insufficient infrastructure, high drug costs, limited provider training, scarce biomarker testing, and urban-rural disparities, all of which collectively restrict the safe and effective utilisation of immunotherapy for the region's growing cancer burden [60].
2. *Insufficient Healthcare Infrastructure:* Despite the promise of immunotherapy in treating advanced cancers, its use in SSA remains extremely limited due to the inadequacy of healthcare infrastructure [64].
3. *Patient Selection and Treatments:* As a result, late-stage cancer patients often lack effective treatment options, contributing to rising cancer mortality and an increasing overall cancer burden in the region. The study carried out by Olatunji et al. [63] is one of the few studies on cancer immunotherapy on the continent, revealing that only 44% of clinics test for PD-L1 expression, and 12% use genomic sequencing to assess tumour mutational burden, which is critical for predicting immunotherapy efficacy. So, at clinics where immunotherapy is practiced, the limited use of these biomarkers means that patient selection is often inadequate, likely reducing treatment effectiveness and safety while increasing the risk of autoimmune side effects due to the inability to accurately predict which patients will benefit from immunotherapy before administration [61, 65, 66].
4. *Financial burden and limited healthcare coverage:* Immunotherapy is an effective treatment for advanced-stage malignancies in SSA clinics, but its wider usage is hampered by high costs and limited healthcare coverage [67, 68]. There is a need to promote access to immunotherapy drugs without a financial burden on cancer patients and their families. Public health initiatives that increase cancer screening and slow disease progression should be introduced. However, boosting therapies effective against advanced cancer is equally critical in slowing the growing cancer-related death rate in SSA [69, 70]. According to recent research, ultra-low dosages of immunotherapy medications may improve survival rates for advanced cancer patients in low- and middle-income nations [71]. Patients may also benefit from immunotherapy clinical trials in SSA, which make the treatment more affordable.
5. *Geographic and Regulatory Barriers:* The majority of cancer centres in SSA are in urban areas, leaving rural communities without access to advanced cancer treatment. Additionally, the underrepresentation of Africans in global immunotherapy studies reduces the availability of region-specific data on therapeutic effectiveness.

It is clear that there are significant hurdles, but despite the hurdles mentioned and more, there is certainly an increase in awareness and efforts to improve cancer care, including the adoption of immunotherapy in SSA.

7. Recommendations: The case for cancer immunotherapy in SSA

To improve and advance cancer treatment and immunotherapy in SSA, we highlighted below some of the recommendations, most of which agree with the

recommendations suggested by Obiozor et al. [72] on feasibility analysis of the preparedness of Africans to access cellular gene therapy. Initial recommendations from Olatunji et al., [63] are that expanded use of immunotherapy in SSA requires improved access to immunotherapy medications, integration of immunotherapy training into ongoing medical education, and enhanced availability and use of biomarker testing to predict immunotherapy response.

Recommendations include:

1. Increased public awareness about the benefits of immunotherapy: Health education on the role of immunotherapy for cancer care should be a top priority for cancer clinics in SSA. This means that individuals are adequately informed of immunotherapy as an improved treatment modality for cancer treatment.
2. Increased accessibility and affordability of cancer immunotherapeutic medications: Subsidies should be granted to cancer patients to increase the adoption of immunotherapeutic medications for cancer treatment in SSA.
3. Training and Education for Clinicians: Clinicians in cancer clinics should be trained on new advances in immunotherapy so that the catalogue of treatment options can be updated in various cancer care clinics across SSA.
4. Addressing infrastructural gap: Infrastructural gap remains a big issue for most healthcare advancements in SSA, leading to less adoption of treatment. Addressing this issue means that governments and other stakeholders invest in infrastructure that is needed for cancer care within SSA. This includes specialised buildings, laboratories, diagnostic centres, and other important infrastructure used within cancer treatment.
5. Integration with the existing cancer control programme.
6. Cost-effective and Collaborative approaches.
7. Utilising predictive markers for pre-screening and implementing immunoprotective treatments to reduce expenses.
8. Bridging knowledge and research gaps, as we have previously discussed in detail.
9. Adequate funding for cancer research in SSA.

8. Conclusion

Cancer treatment has evolved significantly, progressing from traditional approaches to highly specialised and personalised therapies. While surgery, chemotherapy, and radiotherapy remain cornerstones of cancer management, emerging innovations—such as immunotherapy, targeted therapy, and nanotechnology—are transforming the oncology landscape.

For SSA to fully benefit from cancer immunotherapies, several critical challenges must be addressed, including limited clinical infrastructure, a shortage of trained personnel, and barriers to access. It is imperative that the region not only adapt

these advancements but also invest in developing homegrown immunotherapeutic strategies. This will ensure that the growing population of cancer patients across the continent can benefit equitably from these promising frontier therapies.

Conflict of interest

The authors declare no conflict of interest.

Author details

John Oluwafemi Teibo^{1*}, Festus Adebayo Atiba¹, Ayomide Oluwadarasimi Adebisin^{2,3}, Abolaji Samson Olagunju⁴, Titilade Kehinde Ayandeyi Teibo⁵, Ayodeji Mathias Adegoke⁶, Marwan Al-Hajeili⁷ and Gaber El-Saber Batiha⁸

1 Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil

2 Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, USA

3 Harper Cancer Research Institute, University of Notre Dame, Notre Dame, USA

4 Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, Brazil

5 Department of Maternal-Infant and Public Health Nursing, College of Nursing, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

6 Department of Pharmacology, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa

7 Department of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

8 Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour, AlBeheira, Egypt

*Address all correspondence to: johnteibo@usp.br

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] International Agency for Research on Cancer (IARC). Cancer tomorrow. n.d. Available from <https://gco.iarc.fr/en> [Accessed: July 20, 2025]
- [2] The World Health Organization. Cancer. 2025. Available from: <https://www.who.int/news-room/fact-sheets/detail/cancer> [Accessed: May 28, 2025]
- [3] Kaur R, Bhardwaj A, Gupta S. Cancer treatment therapies: Traditional to modern approaches to combat cancers. *Molecular Biology Reports*. 2023;**50**:9663-9676. DOI: 10.1007/s11033-023-08809-3
- [4] Carlotta P, Chiara M, Gianni C. Innovative approaches for cancer treatment: Current perspectives and new challenges. *Ecancermedalscience*. 2019;**13**:961
- [5] Chu DT, Nguyen TT, Tien NLB, Tran DK, Jeong JH, Anh PG, et al. Recent Progress of stem cell therapy in cancer treatment: Molecular mechanisms and potential applications. *Cells*. 2020;**9**(3):563. DOI: 10.3390/cells9030563
- [6] Omotoso OE, Teibo JO, Atiba FA, et al. Bridging the genomic data gap in Africa: Implications for global disease burdens. *Globalization and Health*. 2022;**18**:103. DOI: 10.1186/s12992-022-00898-2
- [7] Teibo JO, Atiba FA, Omotoso OE. Genomics Data Gap in Africa Has Implications for Global Disease Burden. *Nature Africa: Nature Springer*; 2022. DOI: 10.1038/d44148-022-00123-7
- [8] Sullivan R, Alatisie OI, Anderson BO, Audisio R, Autier P, Aggarwal A, et al. Global cancer surgery: Delivering safe, affordable, and timely cancer surgery. *The Lancet Oncology*. 2015;**16**(11):1193-1224
- [9] Mahajan A, Vaish R, Desai S. Current scenario and efforts to propound equal and affordable global cancer surgery. *Indian Journal of Medical and Paediatric Oncology*. 2015;**36**(4):210-211. DOI: 10.4103/0971-5851.171540
- [10] Liu H, Lv L, Yang K. Chemotherapy targeting cancer stem cells. *American Journal of Cancer Research*. 2015;**5**(3):880
- [11] Weeks JC, Catalano PJ, Cronin A, Finkelman MD, Mack JW, Keating NL, et al. Patients' expectations about effects of chemotherapy for advanced cancer. *New England Journal of Medicine*. 2012;**367**(17):1616-1625
- [12] Huang R, Zhou PK. DNA damage repair: Historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. *Signal Transduction and Targeted Therapy*. 2021;**6**(1):254
- [13] Padma VV. An overview of targeted cancer therapy. *Biomedicine*. 2015;**5**:1-6
- [14] Begg AC, Stewart FA, Vens C. Strategies to improve radiotherapy with targeted drugs. *Nature Reviews Cancer*. 2011;**11**(4):239-253
- [15] Zhong L, Li Y, Xiong L, Wang W, Wu M, Yuan T, et al. Small molecules in targeted cancer therapy: Advances, challenges, and future perspectives. *Signal Transduction and Targeted Therapy*. 2021;**6**(1):201. DOI: 10.1038/s41392-021-00572-w
- [16] Chehelgerdi M, Chehelgerdi M, Khorramian-Ghahfarokhi M, et al.

Comprehensive review of CRISPR-based gene editing: Mechanisms, challenges, and applications in cancer therapy. *Molecular Cancer*. 2024;**23**:9. DOI: 10.1186/s12943-023-01925-5

[17] Domka W, Bartusik-Aebischer D, Mytych W, Myśliwiec A, Dynarowicz K, Cieślak G, et al. Photodynamic therapy for eye, ear, laryngeal area, and nasal and oral cavity diseases: A review. *Cancers*. 2024;**16**(3):645

[18] Smith AR. Proton therapy. *Physics in Medicine & Biology*. 2006;**51**(13):R491

[19] Condello M, D'Avack G, Spugnini EP, Meschini S. Electrochemotherapy: An alternative strategy for improving therapy in drug-resistant SOLID tumors. *Cancers*. 2022;**14**(17):4341. DOI: 10.3390/cancers14174341

[20] Song Y, Zou J, Castellanos EA, Matsuura N, Ronald JA, Shuhendler A, et al. Theranostics - A sure cure for cancer after 100 years? *Theranostics*. 2024;**14**(6):2464-2488. DOI: 10.7150/thno.96675

[21] Tagliamonte M, Petrizzo A, Tornesello ML, Ciliberto G, Buonaguro FM, Buonaguro L. Combinatorial immunotherapy strategies for hepatocellular carcinoma. *Current Opinion in Immunology*. 2016;**39**:103-113

[22] Valadi H, Ekström K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology*. 2007;**9**(6):654-659. DOI: 10.1038/ncb1596

[23] Dillman RO. Cancer immunotherapy. *Cancer Biotherapy & Radio-pharmaceuticals*. 2011;**26**(1):1-64. DOI: 10.1089/cbr.2010.0902

[24] Kaptein P, Jacobberger-Foissac C, Dimitriadis P, Voabil P, de Bruijn M, Brokamp S, et al. Addition of interleukin-2 overcomes resistance to neoadjuvant CTLA4 and PD1 blockade in ex vivo patient tumors. *Science Translational Medicine*. 2022;**14**(642):eabj9779. DOI: 10.1126/scitranslmed.abj9779

[25] Salas-Benito D, Pérez-Gracia JL, Ponz-Sarvisé M, Rodríguez-Ruiz ME, Martínez-Forero I, Castañón E, et al. Paradigms on immunotherapy combinations with chemotherapy. *Cancer Discovery*. 2021;**11**(6):1353-1367. DOI: 10.1158/2159-8290.CD-20-1312

[26] Huang J, Reckamp KL. Immunotherapy in advanced non-small cell lung cancer. *Seminars in Respiratory and Critical Care Medicine*. 2020;**41**(3):400-408. DOI: 10.1055/s-0040-1710077

[27] Subudhi SK, Vence L, Zhao H, Blando J, Yadav SS, Xiong Q, et al. Neoantigen responses, immune correlates, and favorable outcomes after ipilimumab treatment of patients with prostate cancer. *Science Translational Medicine*. 2020;**12**(537):eaaz3577. DOI: 10.1126/scitranslmed.aaz3577

[28] Paz-Ares LG, Ramalingam SS, Ciuleanu T-E, Lee J-S, Urban L, Caro RB, et al. First-line Nivolumab plus Ipilimumab in advanced NSCLC: 4-year outcomes from the randomized, open-label, phase 3 CheckMate 227 part 1 trial. *Journal of Thoracic Oncology: Official Publication of the International Association for the Study of Lung Cancer*. 2022;**17**(2):289-308. DOI: 10.1016/j.jtho.2021.09.010

[29] Albiges L, Gurney H, Atduev V, Suarez C, Climent MA, Pook D, et al. Pembrolizumab plus lenvatinib as first-line therapy for advanced non-clear-cell

- renal cell carcinoma (KEYNOTE-B61): A single-arm, multicentre, phase 2 trial. *The Lancet. Oncology*. 2023;**24**(8):881-891. DOI: 10.1016/S1470-2045(23)00276-0
- [30] Addai BW, Ngwa W. COVID-19 and cancer in Africa. *Science* (New York, N.Y.). 2021;**371**(6524):25-27. DOI: 10.1126/science.abd1016
- [31] Kumar M, Thangavel C, Becker RC, Sadayappan S. Monoclonal antibody-based immunotherapy and its role in the development of cardiac toxicity. *Cancers* (Basel). 2020;**13**(1):86. DOI: 10.3390/cancers13010086
- [32] Justiz-Vaillant A, Pandit BR, Unakal C, Vuma S, Akpaka PE. A comprehensive review about the use of monoclonal antibodies in cancer therapy. *Antibodies* (Basel). 2025;**14**(2):35. DOI: 10.3390/antib14020035
- [33] Scott AM, Allison JP, Wolchok JD. Monoclonal antibodies in cancer therapy. *Cancer Immunity*. 2012;**12**:14
- [34] Azoury CS, Straughan MD, Shukla V. Immune checkpoint inhibitors for cancer therapy: Clinical efficacy and safety. *Current Cancer Drug Targets*. 2015;**15**(6):452-462
- [35] Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nature Reviews. Immunology*. 2013;**13**(4):227-242. DOI: 10.1038/nri3405
- [36] Larkin J, Minor D, D'Angelo S, Neyns B, Smylie M, Miller WH, et al. Overall survival in patients with advanced melanoma who received Nivolumab versus investigator's choice chemotherapy in CheckMate 037: A randomized, controlled, open-label phase III trial. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*. 2018;**36**(4):383-390. DOI: 10.1200/JCO.2016.71.8023
- [37] Postow MA, Callahan MK, Wolchok JD. Immune checkpoint blockade in cancer therapy. *Journal of Clinical Oncology*. 2015;**33**(17):1974-1982
- [38] Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. *Nature Communications*. 2020;**11**(1):3801. DOI: 10.1038/s41467-020-17670-y
- [39] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *The New England Journal of Medicine*. 2010;**363**(8):711-723. DOI: 10.1056/NEJMoa1003466
- [40] Li B, Chan HL, Chen P. Immune checkpoint inhibitors: Basics and challenges. *Current Medicinal Chemistry*. 2019;**26**(17):3009-3025. DOI: 10.2174/0929867324666170804143706
- [41] Conlon KC, Miljkovic MD, Waldmann TA. Cytokines in the treatment of cancer. *Journal of Interferon & Cytokine Research*. 2019;**39**(1):6-21. DOI: 10.1089/jir.2018.0019. Epub 2018 Jun 11
- [42] Yang M, Olaoba OT, Zhang C, Kimchi ET, Staveley-O'Carroll KF, Li G. Cancer immunotherapy and delivery system: An update. *Pharmaceutics*. 2022;**14**(8):1630. DOI: 10.3390/pharmaceutics14081630
- [43] Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: What we know so far. *Nature Reviews. Clinical Oncology*. 2023;**20**(6):359-371. DOI: 10.1038/s41571-023-00754-1

- [44] Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: A phase 1 dose-escalation trial. *Lancet* (London, England). 2015;**385**(9967):517-528. DOI: 10.1016/S0140-6736(14)61403-3
- [45] Zhang P, Zhang G, Wan X. Challenges and new technologies in adoptive cell therapy. *Journal of Hematology & Oncology*. 2023;**16**(1):97. DOI: 10.1186/s13045-023-01492-8
- [46] Dagher OK, Schwab RD, Brookens SK, Posey AD. Advances in cancer immunotherapies. *Cell*. 2023;**186**(8):1814-1814.e1. DOI: 10.1016/j.cell.2023.02.039
- [47] Teibo JO, Picanço-Castro V, de Souza LEB, Faça VM. A proteomics outlook on the molecular effectors of CAR-T cell therapy in cancer management. *Journal of Proteome Research*. 2025;**24**(6):2571-2583. DOI: 10.1021/acs.jproteome.4c00930
- [48] Liu G, Rui W, Zhao X, Lin X. Enhancing CAR-T cell efficacy in solid tumors by targeting the tumor microenvironment. *Cellular & Molecular Immunology*. 2021;**18**(5):1085-1095
- [49] Marofi F, Motavalli R, Safonov VA, Thangavelu L, Yumashev AV, Alexander M, et al. CAR T cells in solid tumors: Challenges and opportunities. *Stem Cell Research & Therapy*. 2021;**12**(1):81
- [50] Sterner RC, Sterner RM. CAR-T cell therapy: Current limitations and potential strategies. *Blood Cancer Journal*. 2021;**11**(4):69
- [51] ISCT Telegraft Hub, From the Editors: Cell and Gene Therapy Approvals in 2024. Available from: <https://www.isctglobal.org/telegrafthub/blogs/ken-ip1/2025/01/16/cell-gene-therapy-approvals-i-2024> [Accessed 12 June, 2025]
- [52] Chiocca Antonio E. Oncolytic viruses. *Nature Reviews Cancer*. 2002;**2**(12):938-950
- [53] Pol J, Kroemer G, Galluzzi L. First oncolytic virus approved for melanoma immunotherapy. *Oncoimmunology*. 2016;**5**(1):e1115641
- [54] Ma R, Li Z, Chiocca EA, Caligiuri MA, Yu J. The emerging field of oncolytic virus-based cancer immunotherapy. *Trends in Cancer*. 2023;**9**(2):122-139. DOI: 10.1016/j.trecan.2022.10.003
- [55] Todo T, Ito H, Ino Y, Ohtsu H, Ota Y, Shibahara J, et al. Intratumoral oncolytic herpes virus G47Δ for residual or recurrent glioblastoma: A phase 2 trial. *Nature Medicine*. 2022;**28**(8):1630-1639. DOI: 10.1038/s41591-022-01897-x
- [56] Andtbacka RHI, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*. 2015;**33**(25):2780-2788. DOI: 10.1200/JCO.2014.58.3377
- [57] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a Cancer Journal for Clinicians*. 2021;**71**(3):209-249. DOI: 10.3322/caac.21660
- [58] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022:

GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer Journal for Clinicians. 2024;**74**(3):229-263. DOI: 10.3322/caac.21834

[59] Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. International Journal of Cancer. 2019;**144**(8):1941-1953. DOI: 10.1002/ijc.31937

[60] Gouda HN, Charlson F, Sorsdahl K, Ahmadzadeh S, Ferrari AJ, Erskine H, et al. Burden of non-communicable diseases in sub-Saharan Africa, 1990-2017: Results from the global burden of disease study 2017. The Lancet. Global Health. 2019;**7**(10):e1375-e1387. DOI: 10.1016/S2214-109X(19)30374-2

[61] Ngwa W, Addai BW, Adewole I, Ainsworth V, Alaro J, Alatise OI, et al. Cancer in sub-Saharan Africa: A lancet oncology commission. The Lancet. Oncology. 2022;**23**(6):e251-e312. DOI: 10.1016/S1470-2045(21)00720-8

[62] Garg P, Pareek S, Kulkarni P, Horne D, Salgia R, Singhal SS. Next-generation immunotherapy: Advancing clinical applications in cancer treatment. Journal of Clinical Medicine. 2024;**13**(21):6537. DOI: 10.3390/jcm13216537

[63] Olatunji E, Patel S, Graef K, Joseph A, Lasebikan N, Mallum A, et al. Utilization of cancer immunotherapy in sub-Saharan Africa. Frontiers in Oncology. 2023;**13**:1266514. DOI: 10.3389/fonc.2023.1266514

[64] Kmietowicz Z. Tackle cancer in Africa now to prevent catastrophe, say health activists. BMJ (Clinical Research

Ed.). 2007;**334**(7602):1022-1023. DOI: 10.1136/bmj.39215.854016.BE

[65] Mfuh KO, Abanda NN, Titanji BK. Strengthening diagnostic capacity in Africa as a key pillar of public health and pandemic preparedness. PLOS Global Public Health. 2023;**3**(6):e0001998. DOI: 10.1371/journal.pgph.0001998

[66] Sharma K, Sayed S, Saleh M. Promoting best practice in cancer care in sub-Saharan Africa. Frontiers in Medicine. 2022;**9**:950309. DOI: 10.3389/fmed.2022.950309

[67] Asante A, Wasike WSK, Ataguba JE. Health financing in sub-Saharan Africa: From analytical frameworks to empirical evaluation. Applied Health Economics and Health Policy. 2020;**18**(6):743-746. DOI: 10.1007/s40258-020-00618-0

[68] Barrios C, de Lima Lopes G, Yusof MM, Rubagumya F, Rutkowski P, Sengar M. Barriers in access to oncology drugs—A global crisis. Nature Reviews. Clinical Oncology. 2023;**20**(1):7-15. DOI: 10.1038/s41571-022-00700-7

[69] Omotoso O, Teibo JO, Atiba FA, Oladimeji T, Paimo OK, Ataya FS, et al. Addressing cancer care inequities in sub-Saharan Africa: Current challenges and proposed solutions. International Journal for Equity in Health. 2023;**22**(1):189. DOI: 10.1186/s12939-023-01962-y

[70] Teibo JO, Omotoso O, Atiba FA, Oladimeji T, Paimo TK, Ataya FS, et al. Revisiting cancer care inequities in sub-Saharan Africa: Standpoint and recommendations [abstract]. In: Proceedings of the 17th AACR Conference on the Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved; 2024 Sep 21-24. Los

Angeles, CA. Philadelphia (PA): AACR;
Cancer Epidemiol Biomarkers Prev; 2024
Vol. 33(Suppl. 9): Abstract nr A028

[71] Patil VM, Noronha V, Menon N,
Rai R, Bhattacharjee A, Singh A, et al.
Low-dose immunotherapy in head
and neck cancer: A randomized study.
Journal of Clinical Oncology: Official
Journal of the American Society of
Clinical Oncology. 2023;**41**(2):222-232.
DOI: 10.1200/JCO.22.01015

[72] Obiozor CC, Day LW,
Dube B, Edrah IE, Uzoka E, June CH,
et al. Cellular gene therapy access in
Africa: a multifactorial feasibility
analysis for implementation.
Cytotherapy. 2025;**27**(9):1085-1093. DOI:
10.1016/j.jcyt.2025.04.056

HLA-Based Immunotherapy for Cancer

Lisa M. James

Abstract

Globally, cancer remains a major health concern and is a leading cause of death. Recent advances in cancer immunotherapy, which is aimed at unleashing an individual's immune system to attack cancer, have proven instrumental for improving outcomes in cancer patients, yet limitations of current approaches including resistance, toxicity, and prohibitive costs have led to calls for the development of novel immunotherapeutic strategies to target and eliminate cancer. Following an overview of current cancer immunotherapies, this chapter shines a spotlight on the therapeutic potential of novel interventions centered on human leukocyte antigens (HLA). HLA plays a vital role in immune surveillance and in activating the immune system response to cancer by binding and transporting peptides of cancer antigens to the cell surface to stimulate immune system responses. Indeed, most cancer immunotherapies depend on HLA; yet, individual differences in HLA are often overlooked. The effectiveness of HLA-dependent cancer immunotherapies is partially reliant on tumor antigen peptide-HLA (pHLA) complexes with sufficient binding affinity and immunogenicity to elicit immune system responses aimed at attacking cancer. Since each individual carries a limited repertoire of HLA which determine the landscape of peptides that can be bound and presented, individual variation in HLA takes center stage in the fight against cancer. Strategies focused squarely on optimizing pHLA matches are discussed and novel individually-tailored HLA-based immunotherapeutic strategies are introduced.

Keywords: cancer, immunotherapy, human leukocyte antigen, binding, immunogenicity

1. Introduction

Cancer is a major global health concern and among the leading causes of death worldwide [1, 2]. In 2022, 20 million new cancer cases and nearly 10 million cancer deaths were reported with lung and breast cancer leading the way for cancer-related mortality in men and women, respectively [1]. In 2025, more than 2 million new cancer cases are projected to occur in the United States alone [3] and more than 35 million new cases are predicted worldwide in 2050 [1]. Although cancer incidence remains high [4], relative survival has improved over the last several

decades [5], particularly in high sociodemographic index countries [4]. Treatment advances have significantly contributed to survival outcomes. Traditionally, surgery, chemotherapy, and radiation were the typical standard of care; however, the potential of immunotherapy, which harnesses an individual's own immune system to attack cancer cells, is increasingly recognized as an important tool for cancer treatment, either alone or as an adjunct to standard treatment [6–8]. In this chapter, we briefly review the role of the immune system in cancer, describe current cancer immunotherapies, highlight the central role of human leukocyte antigens (HLA) in the immune response to cancer and on treatment outcomes, and discuss emerging frontiers with regard to novel HLA-based immunotherapeutic strategies.

2. Fundamentals of the immune system

The human immune system plays a crucial role in fighting against cancer via complex interactions between two primary facets referred to as innate immunity and adaptive immunity. Broadly, these separate arms work in concert to protect against invaders including cancer by recognizing and targeting non-self antigens for elimination [9]. The innate immune system is the body's first line of defense that provides a non-specific response that is activated quickly in response to any foreign pathogen. Cells involved in innate immunity include macrophages, phagocytes, neutrophils, natural killer (NK) cells, and dendritic cells (DCs) which are the interface between innate and adaptive immunity. In contrast, the adaptive immune system is slower but provides specific defense against pathogens including development of antibodies and immunological memory. Cells involved in adaptive immunity include cytotoxic CD8 T cells, CD4⁺ helper T cells, and B cells. HLA, also referred to as major histocompatibility complex (MHC), are a central component of the adaptive immune response due to their vital role in presenting tumor antigens to T cells to stimulate an immune response aimed at cancer elimination, as described below (see Section 4).

Details about the roles and interplay of various components of the innate and adaptive immune system in cancer are reviewed elsewhere [10, 11]. Briefly, in immunocompetent individuals and in early stages of tumor development, immunogenic cancer cells—that is, cells that are able to stimulate an immune system response—are detected and eliminated via innate and adaptive immune surveillance and responses [12, 13]. If successful, the individual remains cancer free. However, if cancer cells remain, they may enter equilibrium, a state during which cancer cells are present but further growth is immunologically restrained. Equilibrium, which may be life-long, is strictly a function of adaptive immunity [12]. Disruptions in equilibrium, due to cancer-induced immunosuppression, inflammation, properties of the tumor microenvironment, or selection of less immunogenic cancer cells promote escape and proliferation which ultimately results in disease [10, 13]. During this phase, engagement of immune checkpoints and recruitment of regulatory T cells (Tregs) suppress anti-tumor activity allowing the cells to evade destruction by the immune system, thereby permitting unchecked growth. The aim of immunotherapy is to modify the immune response to promote tumor rejection and reduce tumor promotion, optimally returning the patient to a state of elimination or equilibrium [14, 15]. T cells are at the very center of cancer immunotherapy [16].

3. Cancer immunotherapy

Advances in cancer immunotherapy have exploded in recent years; however, evidence supporting the power of the immune system in cancer treatment dates back to the 1800s when tumor shrinkage was documented in individuals intentionally infected with various bacteria [17]. Perhaps most well-known among immunotherapy pioneers is William B. Coley who is widely considered one of the fathers of immunotherapy. In the late 1800s Coley injected patients with a mixture of live and attenuated bacteria known as “Coley’s toxin” in order to promote strong, anti-tumor immune responses against bone sarcomas, an intervention which resulted in durable remission for many patients [17]. Despite remarkable success, immunotherapy fell out of favor in the wake of the development of radiation and chemotherapy which, along with surgery, became the standard of care. Now, more than a century after Coley’s initial immunotherapy interventions, there has been a resurgence of interest in the promise of cancer immunotherapy, buoyed by enhanced understanding of the immune system’s role in surveillance and elimination of cancer cells. This groundswell has led to the development of several immunotherapeutic strategies that share a goal of unleashing the immune system to attack cancer cells, albeit via different mechanisms. The primary types of immunotherapy are briefly reviewed below.

3.1 Monoclonal antibodies (mAbs)

Antibodies, also known as immunoglobulins, are naturally occurring proteins produced by B cells that bind with and neutralize specific antigens. Monoclonal antibodies (mAbs) are antibodies that have been manufactured in a lab to bind a specific antigen for various therapeutic applications [18]. Over the last several decades, the use of mAbs has exploded with nearly half of all therapeutic antibodies approved for use in the United States or European Union used for cancer treatment (Antibody society) [19]. Several mechanisms of action detailed elsewhere [20, 21] are associated with therapeutic mAbs. They include blocking ligands to prevent signals that promote tumor growth and binding with tumor antigens, triggering (1) antibody-dependent cell cytotoxicity via NK cells, (2) antibody-dependent cell phagocytosis, (3) apoptosis directly, or (4) complement dependent cytotoxicity. Still, others bind with antigens on antigen-presenting cells, acting as an agonist to increase a patient’s T-cell immune response or bind with multiple targets allowing activation of more immune cells to enhance cancer elimination. Finally, others bind with immune checkpoints, a method that has been so influential in cancer treatment that it is covered separately (Section 3.2). Numerous mAbs targeting several different antigens are used clinically in the treatment of cancer [8, 20]. Among them are mAbs targeting CD20, a protein that is abundantly expressed on cancerous B cells in individuals with hematological cancers such as lymphoma or leukemia [22] and mAbs targeting human epidermal growth factor receptor 2 (HER2), which is overexpressed in 20–25% of breast cancers [23] and is found in several other tumor types (e.g., stomach, endometrium, esophagus, etc.) [21]. Treatment with mAbs targeting CD20 and HER2 have been shown to significantly improve survival [22, 24] as have several mAbs targeting other antigens [18, 20]. Nonetheless, significant side effects including tumor lysis syndrome and cytokine release syndrome (CRS) are associated with mAb infusion [18]. Furthermore, innate and/or acquired resistance to mAbs is a major clinical issue resulting in refractory disease in a majority of patients [18, 20]. For a review on mechanisms, therapeutic applications, and limitations of mAbs on cancer and beyond, see [18].

3.2 Immune checkpoint inhibitors (ICIs)

A specific class of mAbs that has shown particular promise involves the blockade of immune checkpoints. Immune checkpoints are proteins on the surface of primarily T-cells that play an important role in regulating immune responses, both stimulating the immune system and inhibiting excessive immune responses that could contribute to autoimmunity. Cancer cells take advantage of inhibitory immune checkpoints by binding with them, suppressing the ability of the T cell to destroy the tumor. Of the numerous checkpoint pairs that have been discovered, the most well-studied are cytotoxic T lymphocyte-associated antigen-4 (CTLA-4)/B7 ligand and programmed cell death protein 1 (PD1)/PD-L1 ligand [25]. ICIs block checkpoint proteins from binding with their partner protein, thereby allowing T cells to destroy tumor cells. Antibodies such as CTLA-4 inhibitors, PD-1 inhibitors, and PD-L1 inhibitors are now widely used as 1st line or 2nd line of treatment for several types of cancer including melanoma, non-small-cell lung cancer, renal cell carcinoma, and bladder cancer, among others [7, 26] with thousands of clinical trials underway [27], many of which are evaluating new pathways and next-generation ICIs [28]. The response rate of single-agent ICI varies tremendously with high (50–80%) response in melanoma to virtually non-existent response for pancreatic cancer [7]. Furthermore, numerous immune-related adverse effects (irAEs) involving multiple organ systems are associated with ICI [29, 30]. A recent review found that 40% of ICI patients experience irAEs and roughly 20% experience high grade adverse events [31], some of which (e.g., myocarditis) are associated with up to 50% mortality [32]. irAEs are even higher for combination ICI [31]. Corticosteroids or other immunosuppressants are often administered to reduce toxicity of ICIs [33], albeit with unclear consequences with regard to cancer outcomes [7]. In light of variable effectiveness and potential toxicity of ICI, there have been calls for innovative treatments with less toxic and more durable effects for cancer treatment [34].

3.3 Adoptive T cell therapy

Adoptive T cell therapy involves collecting and manipulating a patient's T cells to more effectively recognize and kill cancer cells. Two primary strategies, reviewed elsewhere [8, 35], include infusion of tumor-infiltrating lymphocytes (TILs) obtained from a resected tumor and grown in large quantities *ex vivo* or infusion of T cells that are genetically engineered to express either a synthetic chimeric antigen receptor (CAR) that recognizes cell-surface tumor antigens or a T-cell receptor (TCR-T) that recognizes intracellular tumor antigens. Several CAR-T cell therapies are approved for hematological cancers although they are very costly [36, 37] and several challenges limit their efficacy including poor CAR-T cell persistence and cancer cell resistance resulting from antigen loss or modulation [38]. Furthermore, adverse effects are common with 50–95% of patients on CAR-T cell treatments experience cytokine release storm and other adverse effects [35], and up to 50% requiring critical care, vasopressors, or ventilation [6]. Comparatively, TCR-T has a better safety profile due to its MHC restriction [8, 39] and has demonstrated varying degrees of success with synovial sarcoma, melanoma, and human papilloma virus (HPV)-associated cancers [35]; however, severe neurologic toxicities are fairly common and cross-reactivity of TCR-T with normal tissues is a concern [40]. TIL therapy, which relies on a patient's natural T cells, is safer than synthetic CAR-T or TCR-T therapies and has an advantage of the ability to recognize and target several tumor associated antigens (TAAs) compared to the limited number of TAAs targeted by CAR-T or TCR-T [8]. In 2024, the Food and Drug Administration (FDA) approved the first TIL, lifileucel

(Amtagvi), for the treatment of melanoma and has granted Breakthrough Therapy Designation for TILs in treating advanced cervical cancer. Though promising, production of TILs takes several weeks and is costly; consequently, TILs are typically recommended as a second line of therapy or as adjunctive to ICI which is much more readily available and cost-effective [41].

3.4 Vaccines

Cancer vaccines broadly fall into two categories, preventive and therapeutic. Primary preventive vaccines are aimed at preventing the development of cancer, typically by arming the immune system to fight infection with viruses that have been linked to cancer such as HPV and hepatitis B. They are akin to traditional vaccines in which the immune system is primed to attack future occurrence of a given antigen. Preventive vaccines for non-viral cancer antigens including proto-oncogenes are being developed as are vaccines for secondary and tertiary prevention, as reviewed elsewhere [42]. Here, the focus is on therapeutic vaccines. A number of therapeutic vaccines have been developed and are extensively discussed in several recent reviews [43–48]. Briefly, therapeutic cancer vaccines target tumor specific antigens (TSAs) which are uniquely found on tumor cells (e.g., viral antigens, neoantigens) [49–51] or TAAs which may be preferentially found or overexpressed in tumors but may also be present in other cells (e.g., HER2, cancer germline antigens/cancer testis antigens [CTAs]). Furthermore, the antigens may be shared, i.e., common across many individuals with the same type of cancer, or personalized. Most research on therapeutic cancer vaccines to date has focused on shared antigen vaccines although technological advances in sequencing and bioinformatics have led to increased emphasis on the development of personalized vaccines [52].

In addition to the type of antigen targeted, therapeutic cancer vaccines can be distinguished by vaccine platform including cell-based, viral- or bacteria-based, peptide-based, and nucleic acid-based vaccines. Cell-based vaccines include the use of (1) attenuated or lysed tumor cells or (2) antigen presenting cells (APCs)—typically DCs pulsed with tumor antigens—to stimulate T cells responses aimed at tumor elimination. Sipuleucel-T (Provenge), a DC-based vaccine used to treat prostate cancer in the only cell-based vaccine that has been approved by the FDA [53] although numerous cell-based vaccines are under investigation [46]. In addition to preventive virus-based vaccines mentioned previously, therapeutic virus-based cancer vaccines include use of viruses (e.g., adenoviruses, herpes viruses) modified to carry tumor proteins, which when injected into the tumor, selectively replicates within the tumor, prompting cell lysis and activation of the adaptive immune system to develop anti-tumor immunity. Talimogene laherparepvec (T-VEC, Imlygic) which includes herpes simplex virus-1 (HSV-1), is approved for the treatment of advanced melanoma [54]. Viruses can also be used as vectors to deliver antigen-encoding instructions into host cells, prompting protective immune responses, a tactic utilized in recent adenovirus-based vaccine formulations [55]. Although not as widely used, bacteria-based vaccines are promising as a cancer immunotherapy. Bacille Calmette-Guerin (BCG) has been widely utilized for decades to treat noninvasive bladder cancer and several other bacteria-based therapeutic vaccines are in development [56]. Peptide-based vaccines present protein fragments, or subunits, of cancer antigen epitopes (TAAs or TSAs) to APCs. Those peptides bind with MHC molecules and are presented to T cells which recognize the peptides as foreign, thereby stimulating immune responses aimed at their eradication. Synthetic long peptides (11–30 amino acids) have shown particular

promise as they are internalized and processed by APCs to permit binding by both Class I and Class-II HLA molecules, triggering both CD8+ and CD4+ T cell responses [57]. Numerous antigens have been targeted and tested for peptide-based cancer vaccines with minimal adverse effects albeit modest efficacy with regard to survival [8, 57]. Identification of optimal antigens as well as use of adjuvants and nanoparticles aimed at enhancing the immune response are active areas of investigation in the numerous peptide-based cancer vaccine clinical trials underway [8, 46, 57]. Finally, nucleic acid-based vaccines deliver genetic instructions for the production of cancer antigens that, once manufactured, load onto HLA-I and -II molecules, stimulating CD8+ and CD4+ T cells to kill cancer cells and produce antitumor immunity. Detailed information on the attributes and mechanisms of nucleic acid-based vaccines for cancer can be found in a thorough recent review [58]. Nucleic-acid based vaccines encode for specific antigens and can encode multiple antigens that can be personalized. They are also safe and are relatively cost-effective, proving to be a revolutionary addition to cancer immunotherapy, either alone or in combination with other treatments. Although none have been approved for use in cancer to date, dozens are under investigation in clinical trials for several types of cancer including Phase III clinical trials for cervical cancer, melanoma, and colorectal cancer [58, 59]. A critical factor in the effectiveness of nucleic acid-based vaccines is the selection of antigens that are immunogenic. Indeed, a limitation of this approach thus far has been weak and/or lack of sustained immunogenicity [60]. Tremendous ongoing effort is dedicated to optimizing nucleic acid-based cancer vaccines to unleash their potential in eradicating cancer including identification of antigens that are able to bind with lymphocytes (i.e., T cells and B cells) to stimulate a sufficient immune response to attack and eliminate cancer without triggering autoimmune reactions [46, 58].

3.5 Immunotherapy challenges

There is no doubt that recent advances in cancer immunotherapy have reshaped the treatment landscape and improved survival and quality of life for cancer patients [6]. Indeed, >50% 5-year survival has been documented in patients with metastatic melanoma treated with combined ICI [61], a remarkable feat for a condition with historically low survival. Nonetheless, several challenges exist. Depending on the type of immunotherapy, challenges include toxicity and IrAEs that have been associated with significant morbidity and mortality, immune tolerance, primary or secondary (acquired) resistance to immunotherapy, pre-existing immunity, and time-consuming and/or costly manufacturing [7, 8]. To overcome these challenges, ongoing research is aimed at manipulating the tumor microenvironment, identifying patient and cancer markers that predict treatment response and/or resistance, optimizing combination immunotherapy strategies, and leveraging immunology, bioinformatics, and sequencing to identify optimal antigens to generate a just-right immune response. The existing challenges have led to calls for treatments tailored to unique host-cancer pairings [7] and innovative antigen-specific strategies that are less toxic and exploit T-cell memory to improve sustained immunotherapeutic effects [34]. HLA-centric strategies may be just what is needed.

4. HLA: The basics

HLA are cell-surface glycoproteins that play a critical role in human immune surveillance and response. They are also commonly known as MHC given their role in

determining compatibility or rejection in organ or tissue transplants [62]. Their primary function is to present antigens to lymphocytes in order to identify and facilitate immune system activation against foreign and malignant invaders [63]. There are two main classes of HLA—Class I and Class II—that are involved in antigen presentation to T cells. Detailed descriptions of the complex pathways can be found elsewhere [64]; a brief overview is provided here.

HLA Class I (HLA-A, HLA-B, and HLA-C), which are expressed on the surface of all nucleated cells including tumors, present small (8–10 amino acid residues) endogenous antigen peptides to cytotoxic CD8⁺ T cells to signal cell destruction. Specifically, an endogenous antigen is proteolyzed into peptide fragments that are transferred from the cytoplasm to the endoplasmic reticulum with the aid of transporter protein, TAP. Class I molecules synthesized in the endoplasmic reticulum then bind with high affinity antigen peptides forming a pHLA complex that is exported through the Golgi apparatus to the cell surface. When cytotoxic CD8⁺ T cell receptors recognize non-self antigen peptides, they facilitate cell death via release of cytotoxic substances and stimulation of apoptosis.

HLA Class II (HLA-DR, HLA-DQ, HLA-DP), which are expressed on professional APCs including macrophages, B cells, and DCs, present larger (15–22 amino acid residues) exogenous antigens to CD4⁺ helper T cells to stimulate production of antibodies and immunological memory. In the case of HLA Class II, exogenous (i.e., extracellular) antigens are endocytosed or phagocytosed by APCs and, similar to HLA Class-I, are cleaved by proteases into peptide fragments that are transferred to a specialized endosomal antigen loading compartment. HLA Class II molecules are synthesized in the endoplasmic reticulum along with an invariant chain that stabilizes the molecule, prohibits binding endogenous peptides, and facilitates export to the antigen loading compartment where the invariant chain is replaced with a high-affinity antigen peptide, forming a pHLA complex. The pHLA complex is transported via the Golgi apparatus to the cell surface for presentation to CD4⁺ helper T cells which stimulates antigen-specific B-cell mediated antibody production. The antibodies neutralize the antigen and/or stimulate other immune system processes aimed at elimination of the target antigen. Some B cells may be differentiated into memory B cells which help provide durable immune system responses in the event of future exposure to a given antigen, the very mechanism that underlies prophylactic vaccination. Some APCs such as DCs are also able to cross-present internalized exogenous antigens to HLA Class I molecules in which case cytotoxic CD8⁺ T cells and helper CD4⁺ T cells are activated.

The HLA region is the most highly polymorphic in the human genome [65] with over 40,000 HLA alleles already identified and new sequencing techniques (e.g., next generation sequencing, third generation sequencing) contributing to the identification of more novel alleles [66, 67]. This extreme polymorphism at the population level helps protect against diverse pathogens. Each individual, however, carries only 12 classical HLA alleles including 2 from each Class I gene (HLA-A, HLA-B, and HLA-C) and 2 from each Class II gene (HLA-DR, HLA-DQ, HLA-DP), that code for the HLA molecules each individual possesses. Most of the variability across HLA polymorphisms is located in the binding groove [68] which is composed of two alpha domains in the case of HLA Class I and an alpha and beta chain in HLA Class II. The properties of the binding groove (e.g., amino acid sequence, structure) determine which antigens can bind with sufficient affinity to promote an immune response. High binding affinity is necessary to promote an immune response [69]. A single amino acid substitution alters the binding groove [70–72] and thus the repertoire of peptides that can

be bind with HLA for presentation to T cells [73]. Thus, a given individual's immune response, whether it be to bacterial or viral infections or to cancer, is determined by a combination of their HLA composition and the pool of antigen peptides to which their HLA molecules can bind. A single pMHC complex triggers T cell activation [74]. Thus, the immune system is designed to readily act against invaders including cancer; the success, however, rests on high affinity antigen peptide-HLA binding as a starting point for immunogenic and antigenic responses. This point has significant implications for cancer immunotherapy as discussed below.

4.1 HLA and cancer

The importance of HLA in the course of cancer cannot be overstated. Under optimal conditions, tumor antigens are recognized and eliminated by the immune system, prohibiting their further proliferation. If, however, cancer cells escape immune surveillance, tolerance to the cancer antigens can develop permitting unchecked growth. This transition from tumor antigen elimination to escape is commonly referred to as cancer immunoediting [12]. Viewing cancer immunoediting through the lens of HLA, tumor antigens that bind with high affinity to HLA molecules forming an immunogenic pHLA complex are most likely to elicit destruction of cancer cells, promoting survival. On the other hand, antigens with weak affinity and those with low immunogenicity and/or low antigenicity are prone to escape, contributing to morbidity and mortality [12, 75]. One well-established mechanism through which cancer cells escape the immune system is through downregulation of HLA [76]. Thus, HLA binding of tumor antigens plays a critical role in cancer prevention, and when blocked by poor binding, weak immunogenicity/antigenicity, or downregulation, influences cancer progression. Cancer immunotherapies are largely aimed at restoring functional immune system responses in which tumor antigen presentation leads to T-cell mediated immune system activation and elimination of cancer cells. To highlight the critical role of HLA in cancer, consider the following:

1. HLA variation influences the course of cancer. The extremely polymorphic nature of HLA is reflected in allelic variation in HLA-related cancer risk or protection [77–80], response to immunotherapy [81–85], occurrence of IrAEs [76], and metastasis [86].
2. Downregulation of HLA is a common feature in cancer. Downregulation of HLA, loss of HLA, or loss of heterogeneity of HLA is a common feature of cancer immune escape that permits cancer proliferation [86–88]. Depending of the type of cancer, up to 87.9% of cancer cases exhibit abnormalities in HLA/MHC antigen processing machinery [89], some of which are reversible [90]. In addition to antigen processing dysfunction, loss of HLA heterogeneity has also been linked to worse outcomes albeit with some mixed findings [76]. Generally, HLA heterozygosity coupled with high tumor mutational burden are associated with favorable outcomes [77, 83]. Indeed, a diverse pool of HLA and tumor antigens increases the probability of pHLA binding with high affinity and sufficient immunogenicity/antigenicity to stimulate an immune response [91, 92].
3. Interventions aimed at upregulating HLA enhance the effectiveness of T cell immunotherapies for cancer. Various approaches for recovering HLA have been investigated including modulating HLA transcriptional regulators and correct-

ing HLA signaling and trafficking abnormalities [90, 93, 94]. Such interventions have been shown to enhance T cell-mediated immunity and improve the clinical course of the disease [93].

4. Most cancer immunotherapies are HLA-cell dependent. Most cancer immunotherapies including ICIs, adoptive cell therapies such as TILs and TCR-Ts, and cancer vaccines are aimed at unleashing T-cells to enhance an individual's immune response aimed at tumor elimination. HLA binding with tumor antigens is a prerequisite for presentation of tumor antigens to T cells. Although newer HLA-independent therapies (e.g., CAR-T, CAR-NK) are gaining traction, many commonly utilized cancer immunotherapies are at their very core HLA-dependent [95].

4.2 pHLA: The cancer immunotherapy linchpin

This central role of HLA in cancer risk and progression shines a spotlight on the potential for HLA-centric immunotherapies for cancer. On one hand, the relevance of HLA for tumor antigen presentation is well established and, as noted above, recovery of HLA to facilitate antigen presentation is an important immunotherapy tool [90, 93]. However, the mere presence or recovery of HLA may not be sufficient to stimulate an immune system response aimed at cancer elimination, particularly in the case of antigen peptides with weak affinity and/or low immunogenicity/antigenicity to a given HLA molecule. To mount an effective immune response, it is first necessary for tumor antigen peptides to bind with high-affinity to an HLA molecule, forming a peptide-HLA (pHLA) complex. That pHLA complex is then presented to T cells, stimulating an immune response if the complex is sufficiently immunogenic/antigenic. Thus, for each individual, it is the combination of HLA molecules and tumor antigens that is the critical factor in an individual's immune response to cancer, rendering the pHLA complex as an immunotherapy linchpin. Since HLA is extremely diverse and in light of variation in tumor antigens (e.g., shared TAAs, TSAs including person-specific neoantigens), the potential pHLA complex combinations are vast and highly individualized. Recognition of the vital role of high affinity/highly immunogenic and individualized pHLA complexes for cancer immunotherapy has several implications:

1. Treatment aimed at HLA recovery will be ineffective if the individual's HLA do not match their tumor antigens (i.e., pHLA with high affinity, high immunogenicity/antigenicity).
2. HLA-dependent immunotherapy, which includes almost all current immunotherapy approaches, will be of limited benefit in the absence of an HLA-antigen match.
3. Individual variation in HLA and in cancer antigens results in individual differences in (a) ability to bind tumor antigen peptides (pMHC) for presentation to T cells, (b) pMHC complex immunogenicity, and (c) pMHC complex antigenicity. Thus, current off-the-shelf, one-size-fits-all approaches will have mixed effects depending on an individual's HLA-antigen composition.

4.3 Novel HLA-centric strategies to enhance immunotherapies

To maximize the effectiveness of cancer immunotherapies, novel strategies based on HLA-antigen matching will be required. To that end, approaches

combining immunogenetic (i.e., HLA) and tumor sequencing, bioinformatics, and immunology take center stage. Indeed, several bioinformatics-based clinical vaccine trials are recently completed or underway [96] subsequent to promising findings of influential studies demonstrating benefit of personalized vaccines for cancer [97–99]. Innovative approaches that can be implemented quickly and cost-effectively are needed to enhance cancer immunotherapy effectiveness and extend patient survival.

Here, we present several novel HLA-based approaches to cancer immunotherapy (**Figure 1**). One approach is to provide to a patient the blueprint for HLA that will bind with high affinity and immunogenicity to tumor antigens [100]. Since each individual possesses a limited repertoire of HLA alleles, and thus HLA molecules, the likelihood of an individual possessing HLA that that can bind and present tumor antigens to T cells is limited. However, patient-specific antigens can be identified from a tumor and bioinformatics approaches (e.g., INeoEpp [101], NetMHCpan [102]) can be used to determine in silico HLA that are likely to bind with high affinity and sufficient immunogenicity to stimulate an immune response aimed at eliminating the cancer. The “matching” HLA can be synthesized and delivered via vaccination. We [103] recently used an in silico approach to characterize binding affinity and immunogenicity of 2462 HLA Class I alleles to all linear nonamer (9-mer) epitopes of 11 known melanoma antigens. We identified nine HLA Class I alleles that were omnipotent against all 11 antigens, making those HLA ideal candidates for vaccine delivery. Alternatively, HLA-based vaccines could provide HLA that is incompatible with an individual’s HLA composition directly into a tumor [100]. That strategy may lead to rejection of the tumor along the lines of HLA’s role in tissue rejection [62].

An alternative HLA-centric cancer immunotherapy approach is to give to a patient antigen peptides that are predicted to bind with high affinity to the HLA an individual possesses and that are highly immunogenic. Peptide-based vaccines which can target TSAs or TAAs are among the most common cancer immunotherapy approaches [39, 57]. Neoantigen-based TSA approaches have increased exponentially and, in combination with ICI and other immunotherapies, have

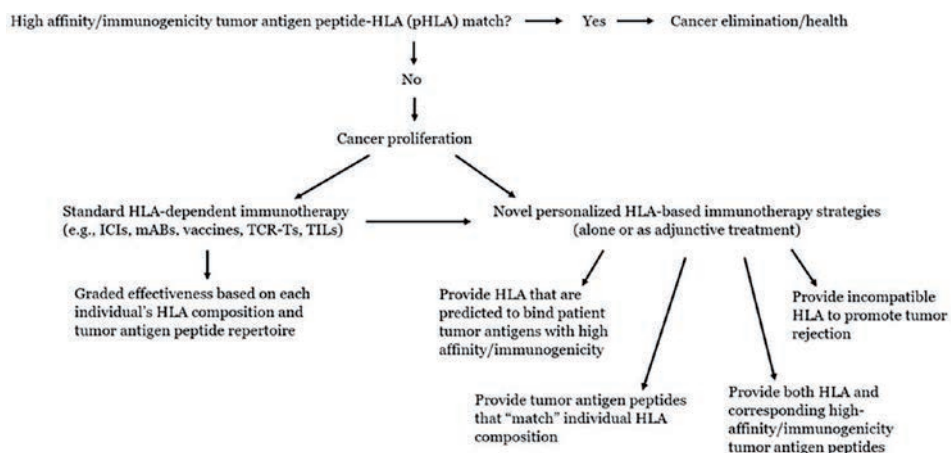


Figure 1. Schematic figure highlighting the central role of HLA in cancer elimination, relevance for standard immunotherapies, and summary of novel HLA-based cancer immunotherapy strategies.

shown promising albeit variable results and are very costly and time-consuming [104]. In contrast, immunotherapies that are based on TAAs are inexpensive and cost-effective since they involve antigens that are often shared across many individuals and are expressed in a wide variety of cancers with the same type of cancer. Among TAAs, cancer germline antigens (also referred to as cancer testis antigens [CTAs]) represent ideal targets for immunotherapy since they are highly immunogenic and are highly expressed in tumors but not in most normal tissues [105, 106]. We have characterized binding affinity and immunogenicity of 6 CTAs with >2000 HLA Class I molecules [103]; as such, specific CTA peptides that match an individual's HLA composition could feasibly be delivered via vaccination. In the absence of a HLA-CTA match, both the HLA blueprint and relevant antigen peptides could be administered via vaccination.

It is worth pointing out that in order to effectively characterize high affinity/high immunogenicity HLA-antigen matches at least 2-field (4-digit) high-resolution HLA typing will be necessary since very small (even single amino acid) differences alter the binding groove and thus the antigens that can bind, influencing disease susceptibility/protection [70, 71]. For example, HLA-DRB1*13:01 and DRB1*13:02 differ by a single amino acid substitution, a seemingly minor difference albeit with major implications for disease susceptibility and outcomes [70, 71, 107]. Thus, sequencing of HLA at the gene (e.g., HLA-DRB1) or allele (HLA-DRB1*13) level will be insufficient for accurately characterizing HLA-antigen affinity, immunogenicity, and antigenicity. Indeed, a single HLA class I point mutation in the HLA class I binding groove has been shown to dramatically enhance cytotoxic T lymphocyte immunotherapy in mice [108]. Furthermore, HLA supertypes [109] and reliance on imputation are replete with their own limitations, discussed elsewhere [110, 111], that are likely to lead to inaccuracies with regard to HLA-antigen matching and limited reproducibility. For instance, the B44 supertype has been associated with favorable ICI outcomes in patients with melanoma [83]; however, immunogenicities of various HLA alleles within the B44 supertype with cancer antigens are heterogeneous [112]. Conversely, the B62 supertype has been associated with poor ICI outcomes for melanoma although the effects have been inconsistent across cohorts [83]. Finally, favorable and detrimental HLA effects on ICI for melanoma have not been replicated in other cancers [81, 113]. Taken together, these considerations point to personalized immunotherapy based on both an individual's HLA composition and tumor antigens as crucial for effective immunotherapy, either alone or as an adjunctive to existing immunotherapies such as ICI.

5. Conclusions

Cancer remains a leading cause of death despite tremendous advances in treatment including the re-emergence and improvement of immunotherapy. Furthermore, many current immunotherapies are associated with a potential for serious adverse effects, are extremely costly, and/or benefit a limited number of patients. Thus, there remains a need for novel cost-effective strategies that maximize enduring benefits across a multitude of cancer patients. Shifting the focus of cancer immunotherapy to individual variation in HLA opens the door to tailored interventions aimed at targeting cancer via pHLA matching. HLA-centric immunotherapies are a new frontier with the potential to transform the landscape of cancer treatment.

Acknowledgements

LMJ thanks Apostolos Georgopoulos for unwavering support and stimulating discussions in collaborative pursuit of HLA-focused discoveries. Partial funding for this work was provided by the University of Minnesota (the Anita Kunin Chair in Women's Healthy Brain Aging) and the U.S. Department of Veterans Affairs. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

Conflict of interest

The author declares no conflict of interest.

Author details

Lisa M. James^{1,2,3}

1 Brain Sciences Center, Department of Veterans Affairs Health Care System, Minneapolis, Minnesota, USA

2 Department of Neuroscience, University of Minnesota Medical School, Minneapolis, Minnesota, USA

3 Department of Psychiatry, University of Minnesota Medical School, Minneapolis, Minnesota, USA

*Address all correspondence to: lmjames@umn.edu

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*. 2024;**74**(3):229-263. DOI: 10.3322/caac.21834
- [2] Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. *Cancer*. 2021;**127**(16):3029-3030. DOI: 10.1002/cncr.33587
- [3] Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. Cancer statistics, 2025. *CA: A Cancer Journal for Clinicians*. 2025;**75**(1):10-45. DOI: 10.3322/caac.21871
- [4] Kocarnik JM, Compton K, Dean FE, et al. Cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life years for 29 cancer groups from 2010 to 2019: A systematic analysis for the global burden of disease study 2019. *JAMA Oncology*. 2022;**8**(3):420-444. DOI: 10.1001/jamaoncol.2021.6987
- [5] Henley SJ, Ward EM, Scott S, et al. Annual report to the nation on the status of cancer, part I: National cancer statistics. *Cancer*. 2020;**126**(10):2225-2249. DOI: 10.1002/cncr.32802
- [6] Ling SP, Ming LC, Dhaliwal JS, et al. Role of immunotherapy in the treatment of cancer: A systematic review. *Cancers*. 2022;**14**:5205. DOI: 10.3390/cancers14215205
- [7] Esfahani K, Roudaia L, Buhlaiga N, et al. A review of cancer immunotherapy: From the past, to the present, to the future. *Current Oncology*. 2020;**27**(Suppl. 2):S87-S97. DOI: 10.3747/co.27.5223
- [8] Liu C, Yang M, Zhang D, Chen M, Zhu D. Clinical cancer immunotherapy: Current progress and prospects. *Frontiers in Immunology*. 2022;**13**:961805. DOI: 10.3389/fimmu.2022.961805
- [9] Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. *Allergy, Asthma and Clinical Immunology*. 2018;**14**(Suppl. 2):49. DOI: 10.1186/s13223-018-0278-1
- [10] Gonzalez H, Hagerling C, Werb Z. Roles of the immune system in cancer: From tumor initiation to metastatic progression. *Genes & Development*. 2018;**32**(19-20):1267-1284. DOI: 10.1101/gad.314617.118
- [11] Abbott M, Ustoyev Y. Cancer and the immune system: The history and background of immunotherapy. *Seminars in Oncology Nursing*. 2019;**35**(5):150923. DOI: 10.1016/j.soncn.2019.08.002
- [12] Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. *Science*. 2011;**331**:1565-1570. DOI: 10.1126/science.1203486
- [13] Teng MW, Galon J, Fridman WH, Smyth MJ. From mice to humans: Developments in cancer immunoediting. *The Journal of Clinical Investigation*. 2015;**125**(9):3338-3346. DOI: 10.1172/JCI80004
- [14] Gubin MM, Vesely MD. Cancer immunoediting in the era of immuno-oncology. *Clinical Cancer Research*. 2022;**28**(18):3917-3928. DOI: 10.1158/1078-0432.CCR-21-1804

- [15] Hiam-Galvez KJ, Allen BM, Spitzer MH. Systemic immunity in cancer. *Nature Reviews. Cancer*. 2021;**21**:345-359. DOI: 10.1038/s41568-021-00347-z
- [16] Oliveira G, Wu CJ. Dynamics and specificities of T cells in cancer immunotherapy. *Nature Reviews. Cancer*. 2023;**23**:295-316. DOI: 10.1038/s41568-023-00560-y
- [17] Oiseth SJ, Aziz MS. Cancer immunotherapy: A brief review of the history, possibilities, and challenges ahead. *Journal of Cancer Metastasis and Treatment*. 2017;**3**:250-261. DOI: 10.20517/2394-4722.2017.41
- [18] Mekala JR, Nalluri HP, Reddy PN, et al. Emerging trends and therapeutic applications of monoclonal antibodies. *Gene*. 2024;**925**:148607. DOI: 10.1016/j.gene.2024.148607
- [19] The Antibody Society. Therapeutic Monoclonal Antibodies Approved or in Review in the EU or US [Internet]. Available from: <http://www.antibodysociety.org/resources/approved-antibodies> [Accessed: May 23, 2025]
- [20] Zahavi D, Weiner L. Monoclonal antibodies in cancer therapy. *Antibodies*. 2020;**9**(3):34. DOI: 10.3390/antib9030034
- [21] Delgado M, Garcia-Sanz JA. Therapeutic monoclonal antibodies against cancer: Present and future. *Cells*. 2023;**12**(24):2837. DOI: 10.3390/cells12242837
- [22] Casan JML, Wong J, Northcott MJ, Opat S. Anti-CD20 monoclonal antibodies: Reviewing a revolution. *Human Vaccines & Immunotherapeutics*. 2018;**14**:2820-2841. DOI: 10.1080/21645515.2018.1508624
- [23] Loibl S, Gianni L. HER2-positive breast cancer. *Lancet*. 2017;**389**:2415-2429. DOI: 10.1016/S0140-6736(16)32417-5
- [24] Swain SM, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: Advances and future directions. *Nature Reviews. Drug Discovery*. 2023;**22**:101-126. DOI: 10.1038/s41573-022-00579-0
- [25] Pardoll D. The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews. Cancer*. 2012;**12**:252-264. DOI: 10.1038/nrc3239
- [26] Shiravand Y, Khodadadi F, Kashani SMA, et al. Immune checkpoint inhibitors in cancer therapy. *Current Oncology*. 2022;**29**:3044-3060. DOI: 10.3390/curroncol29050247
- [27] Wei G, Zhang H, Zhao H, et al. Emerging immune checkpoints in the tumor microenvironment: Implications for cancer immunotherapy. *Cancer Letters*. 2021;**511**:68-76. DOI: 10.1016/j.canlet.2021.04.021
- [28] Marin-Acevedo JA, Kimbrough EO, Lou Y. Next generation of immune checkpoint inhibitors and beyond. *Journal of Hematology & Oncology*. 2021;**14**:1-29. DOI: 10.1186/s13045-021-01056-8
- [29] Martins F, Sofiya L, Sykietis GP, et al. Adverse effects of immune-checkpoint inhibitors: Epidemiology, management and surveillance. *Nature Reviews. Clinical Oncology*. 2019;**16**:563-580. DOI: 10.1038/s41571-019-0218-0
- [30] Johnson DB, Nebhan CA, Moslehi JJ, et al. Immune-checkpoint inhibitors: Long-term implications of toxicity. *Nature Reviews. Clinical Oncology*. 2022;**19**:254-267. DOI: 10.1038/s41571-022-00600-w

- [31] Jayathilaka B, Mian F, Franchini F, Au-Yeung G, IJzerman M. Cancer and treatment specific incidence rates of immune-related adverse events induced by immune checkpoint inhibitors: A systematic review. *British Journal of Cancer*. 2025;**132**:51-57. DOI: 10.1038/s41416-024-02887-1
- [32] Moslehi JJ, Salem JE, Sosman JA, Lebrun-Vignes B, Johnson DB. Increased reporting of fatal immune checkpoint inhibitor-associated myocarditis. *Lancet*. 2018;**391**:933. DOI: 10.1016/S0140-6736(18)30533-6
- [33] Brahmer JR, Lacchetti C, Schneider BJ, et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: American Society of Clinical Oncology Clinical Practice Guideline. *Journal of Clinical Oncology*. 2018;**36**(17):1714-1768. DOI: 10.1200/JCO.2017.77.6385
- [34] Johnson PC, Gainor JF, Sullivan RJ, Longo DL, Chabner B. Immune checkpoint inhibitors—The need for innovation. *The New England Journal of Medicine*. 2023;**388**:1529-1532. DOI: 10.1056/NEJMs2300232
- [35] Brudno JN, Maus MV, Hinrichs CS. CAR T cells and T-cell therapies for cancer: A translational science review. *Journal of the American Medical Association*. 2024;**332**(22):1924-1935. DOI: 10.1001/jama.2024.19462
- [36] Prasad V. Tisagenlecleucel—The first approved CAR-T-cell therapy: Implications for payers and policy makers. *Nature Reviews. Clinical Oncology*. 2018;**15**(1):11-12. DOI: 10.1038/nrclinonc.2017.156
- [37] Dropulić B. CAR-T and cellular gene therapies are too expensive. *Nature Medicine*. 2024;**30**:2714. DOI: 10.1038/s41591-024-03231-z
- [38] Shah NN, Fry TJ. Mechanisms of resistance to CAR T cell therapy. *Nature Reviews. Clinical Oncology*. 2019;**16**:372-385. DOI: 10.1038/s41571-019-0184-6
- [39] He Q, Jiang X, Zhou X, et al. Targeting cancers through TCR-peptide/MHC interactions. *Journal of Hematology & Oncology*. 2019;**12**:139. DOI: 10.1186/s13045-019-0812-8
- [40] Zhang J, Wang L. The emerging world of TCR-T cell trials against cancer: A systematic review. *Technology in Cancer Research & Treatment*. 2019;**18**:153303381931068. DOI: 10.1177/1533033819831068
- [41] Kumar A, Watkins R, Vilgelm AE. Cell therapy with TILs: Training and taming T cells to fight cancer. *Frontiers in Immunology*. 2021;**12**:690499. DOI: 10.3389/fimmu.2021.690499
- [42] Graciotti M, Kandalaft LE. Vaccines for cancer prevention: Exploring opportunities and navigating challenges. *Nature Reviews. Drug Discovery*. 2025;**24**:134-150. DOI: 10.1038/s41573-024-01081-5
- [43] Lin MJ, Svensson-Arvelund J, Lubitz GS, et al. Cancer vaccines: The next immunotherapy frontier. *Nature Cancer*. 2022;**3**:911-926. DOI: 10.1038/s43018-022-00418-6
- [44] Hargrave A, Mustafa AS, Hanif A, Tunio JH, Hanif SNM. Recent advances in cancer immunotherapy with a focus on FDA-approved vaccines and neoantigen-based vaccines. *Vaccine*. 2023;**11**:1633. DOI: 10.3390/vaccines11111633
- [45] Mohite P, Yadav V, Pandhare R, et al. Revolutionizing cancer treatment: Unleashing the power of viral vaccines, monoclonal antibodies, and proteolysis-targeting chimeras in the new era of immunotherapy. *ACS*

Omega. 2024;**9**:7277-7295. DOI: 10.1021/acsomega.3c06501

[46] Ruzzi F, Riccardo F, Conti L, et al. Cancer vaccines: Target antigens, vaccine platforms and preclinical models. *Molecular Aspects of Medicine*. 2025;**101**:101324. DOI: 10.1016/j.mam.2024.101324

[47] Zaidi N, Jaffee EM, Yarchoan M. Recent advances in therapeutic cancer vaccines. *Nature Reviews. Cancer*. 2025;**25**:517-533. DOI: 10.1038/s41568-025-00820-z

[48] Zhou M, Tang Y, Xu W, et al. Bacteria-based immunotherapy for cancer: A systematic review of preclinical studies. *Frontiers in Immunology*. 2023;**14**:1140463. DOI: 10.3389/fimmu.2023.1140463

[49] Zhang Z, Lu M, Qin Y, et al. Neoantigen: A new breakthrough in tumor immunotherapy. *Frontiers in Immunology*. 2021;**12**:672356. DOI: 10.3389/fimmu.2021.672356

[50] Jiang T, Shi T, Zhang H, et al. Tumor neoantigens: From basic research to clinical applications. *Journal of Hematology & Oncology*. 2019;**12**:93. DOI: 10.1186/s13045-019-0787-5

[51] Xie N, Shen G, Gao W, et al. Neoantigens: Promising targets for cancer therapy. *Signal Transduction and Targeted Therapy*. 2023;**8**:9. DOI: 10.1038/s41392-022-01270-x

[52] Chakraborty C, Majumder A, Bhattacharya M, et al. The landscape of neoantigens and its clinical applications: From immunobiology to cancer vaccines. *Current Research in Biotechnology*. 2024;**7**:100177. DOI: 10.1016/j.crbiot.2024.100177

[53] Cheever MA, Higano CS. PROVENGE (Sipuleucel-T) in prostate

cancer: The first FDA-approved therapeutic cancer vaccine. *Clinical Cancer Research*. 2011;**17**:3520-3526. DOI: 10.1158/1078-0432.CCR-10-3126

[54] Pol J, Kroemer G, Galluzzi L. First oncolytic virus approved for melanoma immunotherapy. *Oncoimmunology*. 2016;**5**(1):e1115641. DOI: 10.1080/2162402X.2015.1115641

[55] Chavda VP, Bezbaruah R, Valu D, et al. Adenoviral vector-based vaccine platform for COVID-19: Current status. *Vaccine*. 2023;**11**:432. DOI: 10.3390/vaccines11020432

[56] Zhou Y, Wei Y, Tian X, et al. Cancer vaccines: Current status and future directions. *Journal of Hematology & Oncology*. 2025;**18**:18. DOI: 10.1186/s13045-025-01670-w

[57] Buonaguro L, Tagliamonte M. Peptide-based vaccine for cancer therapies. *Frontiers in Immunology*. 2023;**14**:1210044. DOI: 10.3389/fimmu.2023.1210044

[58] Liao HC, Liu SJ. Advances in nucleic acid-based cancer vaccines. *Journal of Biomedical Science*. 2025;**32**:10. DOI: 10.1186/s12929-024-01102-w

[59] Yaremenko AV, Khan MM, Zhen X, Tang Y, Tao W. Clinical advances of mRNA vaccines for cancer immunotherapy. *Med*. 2025;**6**:100562. DOI: 10.1016/j.medj.2024.11.015

[60] Chekaoui A, Garofalo M, Gad B, et al. Cancer vaccines: An update on recent achievements and prospects for cancer therapy. *Clinical and Experimental Medicine*. 2024;**25**:24. DOI: 10.1007/s10238-024-01541-7

[61] Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Five-year survival with

combined nivolumab and ipilimumab in advanced melanoma. *The New England Journal of Medicine*. 2019;**381**:1535-1546. DOI: 10.1056/NEJMoa1910836

[62] Nakamura T, Shirouzu T, Nakata K, Yoshimura N, Ushigome H. The role of major histocompatibility complex in organ transplantation - donor specific anti-major histocompatibility complex antibodies analysis goes to the next stage. *International Journal of Molecular Sciences*. 2019;**20**:4544. DOI: 10.3390/ijms20184544

[63] Medhasi S, Chantratita N. Human leukocyte antigen (HLA) system: Genetics and association with bacterial and viral infections. *Journal of Immunology Research*. 2022;**2022**:9710376. DOI: 10.1155/2022/9710376

[64] Neefjes J, Jongsma M, Paul P, et al. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nature Reviews. Immunology*. 2011;**11**:823-836. DOI: 10.1038/nri3084

[65] Trowsdale J, Knight JC. Major histocompatibility complex genomics and human disease. *Annual Review of Genomics and Human Genetics*. 2013;**14**:301-323. DOI: 10.1146/annurev-genom-091212-153455

[66] Robinson J, Barker DJ, Georgiou X, Cooper MA, Flicek P, Marsh SGE. IPD-IMGT/HLA Database. *Nucleic Acids Research*. 2020;**48**:D948-D955. DOI: 10.1093/nar/gkz950

[67] Robinson J, Barker DJ, Marsh SGE. 25 years of the IPD-IMGT/HLA database. *Human Leukocyte Antigens*. 2024;**103**:e15549. DOI: 10.1111/tan.15549

[68] Dendrou C, Petersen J, Rossjohn J, et al. HLA variation and disease. *Nature*

Reviews. Immunology. 2018;**18**:325-339. DOI: 10.1038/nri.2017.143

[69] Sette A, Vitiello A, Rehman B, et al. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. *Journal of Immunology*. 1994;**153**:5586-5592

[70] Hov JR, Kosmoliaptsis V, Traherne JA, et al. Electrostatic modifications of the human leukocyte antigen-DR P9 peptide-binding pocket and susceptibility to primary sclerosing cholangitis. *Hepatology*. 2011;**53**:1967-1976. DOI: 10.1002/hep.24299

[71] Davenport MP, Quinn CL, Chicz RM, et al. Naturally processed peptides from two disease-resistance-associated HLA-DR13 alleles show related sequence motifs and the effects of the dimorphism at position 86 of the HLA-DR beta chain. *Proceedings of the National Academy of Sciences of the United States of America*. 1995;**92**:6567-6571. DOI: 10.1073/pnas.92.14.6567

[72] van Deutekom HW, Keşmir C. Zooming into the binding groove of HLA molecules: Which positions and which substitutions change peptide binding most? *Immunogenetics*. 2015;**67**(8):425-436. DOI: 10.1007/s00251-015-0849-y

[73] Paul S, Weiskopf D, Angelo MA, Sidney J, Peters B, Sette A. HLA class I alleles are associated with peptide-binding repertoires of different size, affinity, and immunogenicity. *Journal of Immunology*. 2013;**191**:5831-5839. DOI: 10.4049/jimmunol.1302101

[74] Huang J, Brameshuber M, Zeng X, et al. A single peptide-MHC complex ligand triggers digital cytokine secretion in CD4⁺ T cells. *Immunity*. 2013;**39**:846-857. DOI: 10.1016/j.immuni.2013.08.036

- [75] Beatty GL, Gladney WL. Immune escape mechanisms as a guide for cancer immunotherapy. *Clinical Cancer Research*. 2015;**21**:687-692. DOI: 10.1158/1078-0432.CCR-14-1860
- [76] Jiang N, Yu Y, Wu D, et al. HLA and tumour immunology: Immune escape, immunotherapy and immune-related adverse events. *Journal of Cancer Research and Clinical Oncology*. 2023;**149**:737-747. DOI: 10.1007/s00432-022-04493-1
- [77] Wang QL, Wang TM, Deng CM, et al. Association of HLA diversity with the risk of 25 cancers in the UK Biobank. *eBioMedicine*. 2023;**92**:104588. DOI: 10.1016/j.ebiom.2023.104588
- [78] James LM, Georgopoulos AP. Breast cancer, viruses, and human leukocyte antigen (HLA). *Scientific Reports*. 2024;**14**:16179. DOI: 10.1038/s41598-024-65707-9
- [79] James LM, Georgopoulos AP. Immunogenetic profiles and associations of breast, cervical, ovarian, and uterine cancers. *Cancer Informatics*. 2023;**22**:11769351221148588. DOI: 10.1177/11769351221148588
- [80] Georgopoulos AP, James LM. Immunogenetic association of 127 human leukocyte antigen (HLA) alleles with 30 cancers in continental Western European countries. *Journal of Immunological Sciences*. 2022;**6**(2):6-17. DOI: 10.29245/2578-3009/2022/2.1227
- [81] Naranbhai V, Viard M, Dean M, et al. HLA-A*03 and response to immune checkpoint blockade in cancer: An epidemiological biomarker study. *The Lancet Oncology*. 2022;**23**:172-184. DOI: 10.1016/S1470-2045(21)00582-9
- [82] Sabbatino F, Liguori L, Polcaro G, et al. Role of human leukocyte antigen system as a predictive biomarker for checkpoint-based immunotherapy in cancer patients. *International Journal of Molecular Sciences*. 2020;**21**:7295. DOI: 10.3390/ijms21197295
- [83] Chowell D, Morris LGT, Grigg CM, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science*. 2018;**359**:582-587. DOI: 10.1126/science.aao4572
- [84] Amrane K, Le Meur C, Besse B, et al. HLA-DR expression in melanoma: From misleading therapeutic target to potential immunotherapy biomarker. *Frontiers in Immunology*. 2024;**14**:1285895. DOI: 10.3389/fimmu.2023.1285895
- [85] Schaafsma E, Fugle CM, Wang X, et al. Pan-cancer association of HLA gene expression with cancer prognosis and immunotherapy efficacy. *British Journal of Cancer*. 2021;**125**:422-432. DOI: 10.1038/s41416-021-01400-2
- [86] Seliger B, Kloor M, Ferrone S. HLA class II antigen-processing pathway in tumors: Molecular defects and clinical relevance. *Oncoimmunology*. 2017;**6**(2):e1171447. DOI: 10.1080/2162402X.2016.1171447
- [87] Garrido F, Aptsiauri N. Cancer immune escape: MHC expression in primary tumours versus metastases. *Immunology*. 2019;**158**(4):255-266. DOI: 10.1111/imm.13114
- [88] Campoli M, Ferrone S. HLA antigen changes in malignant cells: Epigenetic mechanisms and biologic significance. *Oncogene*. 2008;**27**:5869-5885. DOI: 10.1038/onc.2008.273
- [89] Cai L, Michelakos T, Yamada T, et al. Defective HLA class I antigen processing machinery in cancer. *Cancer Immunology, Immunotherapy*.

2018;**67**(6):999-1009. DOI: 10.1007/s00262-018-2131-2

[90] Hazini A, Fisher K, Seymour L. Deregulation of HLA-I in cancer and its central importance for immunotherapy. *Journal for Immunotherapy of Cancer*. 2021;**9**(8):e002899. DOI: 10.1136/jitc-2021-002899

[91] Pierini F, Lenz TL. Divergent allele advantage at human MHC genes: Signatures of past and ongoing selection. *Molecular Biology and Evolution*. 2018;**35**(9):2145-2158. DOI: 10.1093/molbev/msy116

[92] Chowell D, Krishna C, Pierini F, et al. Evolutionary divergence of HLA class I genotype impacts efficacy of cancer immunotherapy. *Nature Medicine*. 2019;**25**:1715-1720. DOI: 10.1038/s41591-019-0639-4

[93] Sadagopan A, Michelakos T, Boyiadzis G, Ferrone C, Ferrone S. Human leukocyte antigen class I antigen-processing machinery upregulation by anticancer therapies in the era of checkpoint inhibitors: A review. *JAMA Oncology*. 2022;**8**(3):462-473. DOI: 10.1001/jamaoncol.2021.5970

[94] Aktar N, Yueting C, Abbas M, et al. Understanding of immune escape mechanisms and advances in cancer immunotherapy. *Journal of Oncology*. 2022;**2022**:8901326. DOI: 10.1155/2022/8901326

[95] Wang C, Xiong C, Hsu YC, Wang X, Chen L. Human leukocyte antigen (HLA) and cancer immunotherapy: HLA-dependent and-independent adoptive immunotherapies. *Annals of Blood*. 2020;**5**:14. DOI: 10.21037/aob-20-27

[96] Khaddour K, Buchbinder EI. Individualized neoantigen-directed

melanoma therapy. *American Journal of Clinical Dermatology*. 2025;**26**:225-235. DOI: 10.1007/s40257-025-00920-4

[97] Carreno BM, Magrini V, Becker-Hapak M, et al. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science*. 2015;**348**:803-808. DOI: 10.1126/science.aaa3828

[98] Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature*. 2017;**547**:222-226. DOI: 10.1038/nature23003

[99] Ott PA, Hu Z, Keskin DB, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature*. 2017;**547**(7662):217-221. DOI: 10.1038/nature22991

[100] Georgopoulos AP, James LM. Direct administration of human leucocyte antigen (dHLA) molecules into tumour sites: Proposal for a new immunotherapy for cancer. *BJC Reports*. 2023;**1**:17. DOI: 10.1038/s44276-023-00017-7

[101] Wang G, Wan H, Jian X, et al. INeo-Epp: A novel T-cell HLA class-I immunogenicity or neoantigenic epitope prediction method based on sequence-related amino acid features. *BioMed Research International*. 2020;**2020**:5798356. DOI: 10.1155/2020/5798356

[102] Reynisson B, Alvarez B, Paul S, Peters B, Nielsen M. NetMHCpan-4.1 and NetMHCIIpan-4.0: Improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. *Nucleic Acids Research*. 2020;**48**:W449-W454. DOI: 10.1093/nar/gkaa379

- [103] Georgopoulos AP, James LM, Sanders M. Nine human leukocyte antigen (HLA) class I alleles are omnipotent against 11 antigens expressed in melanoma tumors. *Cancer Informatics*. 2024;**23**:11769351241274160. DOI: 10.1177/11769351241274160
- [104] Niemi JVL, Sokolov AV, Schiöth HB. Neoantigen vaccines; clinical trials, classes, indications, adjuvants and combinatorial treatments. *Cancers*. 2022;**14**:5163. DOI: 10.3390/cancers14205163
- [105] Fan C, Qu H, Wang X, Sobhani N, et al. Cancer/testis antigens: From serology to mRNA cancer vaccine. *Seminars in Cancer Biology*. 2021;**76**:218-231. DOI: 10.1016/j.semcancer.2021.04.016
- [106] Alsalloum A, Shevchenko JA, Sennikov S. The melanoma-associated antigen family a (MAGE-A): A promising target for cancer immunotherapy? *Cancers*. 2023;**15**(6):1779. DOI: 10.3390/cancers15061779
- [107] Furukawa H, Oka S, Tsuchiya N, et al. The role of common protective alleles HLA-DRB1*13 among systemic autoimmune diseases. *Genes and Immunity*. 2017;**18**:1-7. DOI: 10.1038/gene.2016.40
- [108] Matsui M, Kawano M, Matsushita S, Akatsuka T. Introduction of a point mutation into an HLA class I single-chain trimer induces enhancement of CTL priming and antitumor immunity. *Molecular Therapy - Methods & Clinical Development*. 2014;**1**:14027. DOI: 10.1038/mtm.2014.27
- [109] Sidney J, Peters B, Frahm N, Brander C, Sette A. HLA class I supertypes: A revised and updated classification. *BMC Immunology*. 2008;**9**:1. DOI: 10.1186/1471-2172-9-1
- [110] James LM, Georgopoulos AP. Protective and susceptibility effects of human leukocyte antigen on melanoma prevalence and their implications for predicting checkpoint blockade immunotherapy. *Journal of Immunological Sciences*. 2022;**6**(2):25-35. DOI: 10.29245/2578-3009/2022/2.1238
- [111] Vlachopoulou E, Lahtela E, Wennerström A, et al. Evaluation of HLA-DRB1 imputation using a Finnish dataset. *Tissue Antigens*. 2014;**83**(5):350-355. DOI: 10.1111/tan.12343
- [112] Georgopoulos AP, James LM, Charonis SA, Sanders M. Melanoma and human leukocyte antigen (HLA): Immunogenicity of 69 HLA class I alleles with 11 antigens expressed in melanoma tumors. *Cancer Informatics*. 2023;**22**:11769351231172604. DOI: 10.1177/11769351231172604
- [113] Abed A, Calapre L, Lo J, et al. Prognostic value of HLA-I homozygosity in patients with non-small cell lung cancer treated with single agent immunotherapy. *Journal for Immunotherapy of Cancer*. 2020;**8**:e001620. DOI: 10.1136/jitc-2020-001620

Chapter 3

Predictive Biomarkers of Immunotherapy

Laya K. Sathyan, Srinivas Naidu and Piyush Kumar

Abstract

Precision cancer treatment greatly benefits from the use of biomarkers. A predictive biomarker is a marker that indicates the likely response to treatment, in terms of tumor regression or patient survival. Improvements in cancer-related morbidity and mortality for certain malignancies have resulted from advancements in screening, diagnosis, systemic medicines, surgical interventions, and supportive care, with the finding of cancer-specific biomarkers serving a significant contributory role. Cancer biomarkers are often classified into two categories: predictive biomarkers, which reflect the illness's response to various treatments, and prognostic markers, which predict specific disease outcomes. This chapter emphasizes the significance of predictive biomarkers—such as tumor mutational burden (TMB), neutrophil-to-lymphocyte ratio (NLR), programmed death-ligand 1 (PD-L1), mismatch repair (MMR) and microsatellite instability (MSI) status, and tumor-infiltrating lymphocytes (TILs)—in directing precision oncology therapies.

Keywords: immunotherapy, predictive biomarkers, prognostic biomarkers, tumor mutational burden (TMB), neutrophil-to-lymphocyte ratio (NLR), programmed death-ligand 1 (PD-L1), mismatch repair (MMR), microsatellite instability (MSI), tumor-infiltrating lymphocytes (TILs)

1. Introduction

Since the introduction of ipilimumab in 2011, immunotherapy has drastically revolutionized the landscape of cancer treatment, earning the title of best clinical cancer progress by the American Society of Clinical Oncology (ASCO) in 2016 [1]. James P. Allison and Tasuku Honjo's key contributions to immune checkpoint blockade were recognized with the 2018 Nobel Prize in Physiology or Medicine, laying the groundwork for medicines targeting the PD-1, PD-L1, and CTLA-4 pathways [2]. Immune checkpoint inhibitors (ICIs) have recently received FDA clearance for a number of difficult-to-treat malignancies, including non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), and melanoma [3–5]. Unlike typical cytotoxic or targeted therapy, ICIs can produce long-lasting responses, which are occasionally sustained even after discontinuation [3]. However, monotherapy benefits only a subset of patients, with objective responses in fewer than 50%, and combination regimens,

while more effective, come with increased immune-related toxicities and financial burden [6]. As a result, there is an urgent need for predictive biomarkers to optimize treatment decisions and identify patients most likely to benefit. Biomarkers such as tumor mutational burden (TMB), programmed death-ligand 1 (PD-L1) expression, mismatch repair deficiency (dMMR)/microsatellite instability (MSI), neutrophil-to-lymphocyte ratio (NLR), and tumor-infiltrating lymphocytes (TILs) have shown promise in stratifying responses and improving outcomes in immuno-oncology [7–9].

2. Immunotherapy and biomarkers

Biomarkers are objectively measurable indicators of biological or pathogenic processes, or responses to therapeutic interventions. In the context of oncology, biomarkers are critical tools for disease diagnosis, prognosis, and predicting therapeutic response. Cancer biomarkers encompass a wide range of molecular features, including alterations in gene expression (such as microRNAs), epigenetic modifications, chromosomal translocations, gene amplifications, copy number variations, single nucleotide polymorphisms (SNPs), and small insertions or deletions [10]. The ideal biomarker should be highly specific to the pathological state and absent or minimally expressed in normal physiological conditions.

Predictive biomarkers, in particular, can predict treatment effectiveness by identifying patient subpopulations that will benefit from a certain therapy method. In contrast, prognostic biomarkers give information regarding disease development regardless of therapy. The discovery of cancer-specific biomarkers has transformed oncological practice by allowing for early identification, risk stratification, and personalized treatment methods, eventually contributing to better clinical outcomes and lower cancer mortality [11].

In the context of *cancer immunotherapy*, several predictive biomarkers have shown clinical utility. These include the *NLR*, *TMB*, *PD-L1* expression, *dMMR/MSI*, *POLE* mutations, and the presence of “hot tumors”, characterized by an inflamed tumor microenvironment rich in immune cell infiltration [7]. These biomarkers guide therapeutic decisions, particularly in selecting patients ICIs, which target pathways such as PD-1/PD-L1 and CTLA-4.

While predictive biomarkers focus on treatment response, *prognostic biomarkers* remain relevant regardless of the therapy administered. For instance, in HER2-positive metastatic breast cancer, the *PIK3CA* gene mutation has been associated with unfavorable prognosis. The *CLEOPATRA* trial demonstrated that first-line therapy with a combination of trastuzumab, pertuzumab, and docetaxel significantly increased progression-free survival (PFS) compared to placebo, demonstrating the value of biomarker-guided therapy regimens [12].

Immunotherapy has transformed treatment paradigms for a wide range of cancers, autoimmune disorders, and infectious illnesses by boosting the body’s own immune surveillance mechanisms. It works by activating adaptive and innate immune system components such as T cells, dendritic cells, and natural killer (NK) cells, which recognize and remove cancerous cells. Immune checkpoint inhibitors have produced long-term responses in numerous malignancies, although their effectiveness is confined to a subgroup of patients [2]. Furthermore, therapy is frequently accompanied by immune-related adverse events (irAEs), which can range from minor autoimmune signs to fatal toxicities [13]. This heterogeneity in response and safety profiles emphasizes the need of solid predictive biomarkers in optimizing patient selection and reducing unnecessary exposure.

The development of reliable biomarkers remains a complex challenge due to the dynamic and heterogeneous nature of the *tumor microenvironment (TME)*. The mechanisms of action of various immunotherapeutic agents are not fully elucidated, and their targets are often inducible and temporally regulated, further complicating the biomarker discovery process [14]. The TME comprises a network of immune cells—including NK cells, eosinophils, neutrophils, dendritic cells, macrophages, T and B lymphocytes—and non-immune components such as fibroblasts, endothelial cells, adipocytes, and extracellular matrix. These constituents engage in complex, bidirectional interactions that modulate tumor progression and therapeutic resistance [15].

Moreover, spatial and metabolic heterogeneity within tumors leads to the formation of *microniches*—localized areas with distinct oxygenation, nutrient availability, and cellular composition. These niches create immunosuppressive zones that foster the evolution of therapy-resistant clones and contribute to immune evasion. The phenomenon of *immunoediting*, encompassing the stages of elimination, equilibrium, and escape, exerts selection pressure on tumor cells, allowing the emergence of variants that can survive immune attack [16]. Overcoming these barriers through a deeper understanding of immune-tumor interactions and the development of integrated biomarker panels is essential to fully realize the potential of precision immunotherapy.

3. Types of predictive biomarkers

3.1 Tumor mutational burden- (TMB)

Tumor Mutational Burden (TMB) has emerged as a key predictive biomarker for immunotherapy, particularly ICIs. It quantifies the number of somatic mutations per megabase (mut/Mb) of the tumor genome and reflects the likelihood of a tumor generating neoantigens capable of eliciting an immune response. These neoantigens, presented via major histocompatibility complexes (MHCs), enhance tumor immunogenicity, thereby increasing responsiveness to ICIs such as PD-1 or PD-L1 inhibitors [17]. Among various tumor types, lung cancers—especially those related to tobacco exposure—exhibit the highest TMB levels, with smokers showing a median TMB of 10.5 mut/Mb compared to 0.6 mut/Mb in non-smokers [18]. The clinical significance of TMB was highlighted in the CheckMate 026 trial, where patients with advanced non-small cell lung cancer (NSCLC) and high TMB had a higher overall response rate (47% vs. 28%) and longer progression-free survival (9.7 vs. 5.8 months) when treated with nivolumab compared to chemotherapy, highlighting its potential to guide first-line immunotherapy decisions [19]. In a 40-year-old patient with metastatic squamous NSCLC and PD-L1 expression <1%, next-generation sequencing identified TMB >10 mut/Mb. Pembrolizumab monotherapy was started, and after 6 months, the patient had a radiologic response that exceeded partial improvement, demonstrating the therapeutic value of TMB in patient selection.

3.1.1 Clinical scenario

After two courses of chemotherapy, a 40-year-old man with metastatic NSCLC (squamous histology) and low PD-L1 expression had disease progression. The next-generation sequencing (NGS) research indicated a high TMB (TMB > 10 mut/Mb). Based on these findings, the patient was started on pembrolizumab 200 mg IV

every 3 weeks as monotherapy. After 6 months of therapy, the patient showed clinical improvements, with radiologic evidence of a response larger than partial improvement, supporting TMB's therapeutic value as a biomarker.

3.2 Neutrophil to lymphocyte ratio - (NLR)

The neutrophil-to-lymphocyte ratio (NLR) is a simple and affordable biomarker generated from regular complete blood counts that represents the balance of the immune system's innate and adaptive arms. Neutrophils, as part of the innate immune response, play an important role in inflammation and host defense via mechanisms such as chemotaxis, phagocytosis, reactive oxygen species (ROS) production, and inflammatory mediator secretion, whereas lymphocytes, key components of the adaptive immune response, are required for anti-tumor immunity and immune surveillance [20, 21]. An increased NLR, which indicates a relative increase in neutrophils or a reduction in lymphocytes, has been linked to poor outcomes in a range of conditions, most notably cancer and critical care. A high NLR in cancer patients is thought to be a measure of systemic inflammation and immune suppression, both of which promote tumor growth and associated with poor OS and PFS across a variety of tumor types, including lung, colorectal, breast, and gastric cancers [22]. Furthermore, in the context of immunotherapy, especially in patients receiving PD-1/PD-L1 inhibitors, a higher NLR has been associated to worse response rates and poorer survival outcomes, highlighting the tumor's immunosuppressive environment [23]. Beyond oncology, NLR is increasingly being employed as a prognostic marker in sepsis and critical disease. A meta-analysis of septic patients found that greater NLR was substantially linked with an increased mortality risk (hazard ratio 1.75) [24]. A prospective ICU research found that septic patients had a mean NLR of 9.53 ± 2.31 , whereas those with septic shock had an even higher NLR (10.31 ± 2.32). NLR's predictive usefulness for severe sepsis was validated by sensitivity (47%), specificity (78%), and an area under the curve (AUC) of 0.631 ($p < 0.05$). Additionally, presepsin levels and Sequential Organ Failure Assessment (SOFA) scores associated strongly with NLR values ($r = 0.65$) [25]. These results highlight NLR's wide clinical utility as a non-specific but strong biomarker for risk stratification, disease severity, and treatment decision-making across several pathological states.

3.3 Programmed death-ligand 1 (PD-L1)

Programmed death-ligand 1 (PD-L1) is a transmembrane protein found on tumor cells and other immune cells. It regulates immunological checkpoints by attaching to its receptor, programmed cell death protein 1 (PD-1), which is largely expressed on activated T cells. The PD-1/PD-L1 axis is a physiological mechanism that maintains peripheral tolerance and prevents autoimmune responses; however, many cancers exploit this pathway by overexpressing PD-L1 to suppress T-cell activation, induce T-cell exhaustion, and avoid immune-mediated destruction within the tumor microenvironment [26, 27]. The introduction of immune checkpoint inhibition, especially monoclonal antibodies targeting PD-1 or PD-L1, has dramatically altered the landscape of cancer treatment. Pembrolizumab, nivolumab, and cemiplimab—PD-1 inhibitors—as well as PD-L1 inhibitors like atezolizumab, durvalumab, and avelumab—have acquired regulatory clearance for a variety of cancers, including melanoma, NSCLC, renal cell carcinoma, and urothelial carcinoma [28, 29]. Nivolumab, a human IgG4 monoclonal antibody, was originally authorized by the

FDA in December 2014 for the treatment of unresectable or metastatic melanoma and then extended to include metastatic NSCLC in March 2015 [30]. The therapeutic value of PD-L1 expression as a predictive biomarker has been particularly evident in NSCLC, where the tumor percentage score (TPS), which quantifies PD-L1 expression in tumor cells, is used to stratify patients for immunotherapy eligibility. Higher PD-L1 expression (TPS $\geq 50\%$) has been linked to improved results after PD-1/PD-L1 blocking, although responses may still occur at lower expression levels.

3.3.1 Clinical scenario

A 50-year-old man who is a smoker with metastatic lung cancer without identifiable driver mutations had a PD-L1 TPS of 60%. The patient underwent first-line immunotherapy with atezolizumab 1200 mg IV every 3 weeks and had an OS benefit of more than 2 years, demonstrating the therapeutic importance of PD-L1 as a biomarker for treatment selection in immuno-oncology.

3.4 Mismatch repair (dMMR) and microsatellite instability (MSI)

Mismatch repair deficit (dMMR) and microsatellite instability (MSI) are well-established predictors of ICI therapeutic response. The mismatch repair mechanism helps to preserve genomic integrity by fixing base-pair mismatches during DNA replication. Defects in this process, caused by the inactivation or loss of function of genes including MLH1, MSH2, MSH6, and PMS2, result in the accumulation of replication mistakes, especially in short, repeating DNA sequences called microsatellites. This accumulation produces a hypermutated phenotype known as MSI [31].

MSI status may be determined using immunohistochemistry (IHC), which measures the expression of mismatch repair proteins, or molecular methods such as polymerase chain reaction (PCR) and next-generation sequencing (NGS), which directly analyze microsatellite loci [32]. MSI-high (MSI-H) or dMMR status occurs in around 15% of colorectal malignancies (CRC), and it is most typically related with Lynch syndrome or spontaneous MLH1 promoter methylation [33]. These tumors often have increased TILs and a high neoantigen burden, making them more sensitive to immune checkpoint inhibition.

MSI-H/dMMR's therapeutic significance as a predictive biomarker for immunotherapy was initially proven in the KEYNOTE-028 phase I research, in which pembrolizumab, a PD-1 inhibitor, induced persistent responses across a range of MSI-H solid tumors [34]. These results were confirmed in the KEYNOTE-016 phase II study, which compared the outcomes of patients with mismatch repair-proficient (pMMR)/microsatellite stable (MSS) metastatic CRC to those with dMMR/MSI-H tumors. The pMMR/MSS cohort showed no objective responses (ORR 0%), while the dMMR/MSI-H group exhibited an ORR of 50% and a disease control rate (DCR) of 89%. Furthermore, the dMMR/MSI-H group did not achieve median PFS or OS, suggesting a long-term benefit. In comparison, the pMMR/MSS group had a median PFS of 2.4 months and an OS of 6 months [35]. These results identified dMMR/MSI-H as an important biomarker for patient selection in immunotherapy, especially in metastatic CRC.

These results highlight the value of MSI-H/dMMR testing in everyday clinical practice, especially in CRC, where it not only identifies patients who are suitable for immunotherapy but also gives essential prognostic information.

3.4.1 Case scenario

A 50-year-old patient with right-sided metastatic colon cancer and synchronous liver metastases underwent biomarker profiling. Molecular tests confirmed RAS wild-type status and no BRAF mutations. IHC analysis revealed a mismatch repair-deficient phenotype owing to the absence of MSH2 and MSH6 expression. Given the verified dMMR/MSI-H status, the patient began combination immunotherapy with nivolumab and ipilimumab every 3 weeks for 6 cycles, followed by 1 year of maintenance nivolumab monotherapy. The patient had a significant radiologic response, with full clearance of liver metastases and a prolonged partial response at the main tumor location.

3.5 Tumor-infiltrating lymphocytes (TILs)

Tumor-infiltrating lymphocytes (TILs) are immune cells that move into the tumor microenvironment (TME) as part of the host's adaptive immunological response. They include CD4⁺ helper T cells, CD8⁺ cytotoxic T cells, and Tregs. These lymphocytes, which are either inside the tumor core (intratumoral TILs) or in the surrounding stroma (stromal TILs), are thought to be a proxy for the tumor's immunogenicity. Their presence indicates a continuous anti-tumor immune response and has been repeatedly linked to better clinical outcomes in a variety of malignancies, including melanoma, breast cancer, ovarian cancer, and NSCLC [36]. High TIL levels are often linked with a better prognosis and longer life, particularly in "immune-hot" tumors that react better to ICIs targeting the PD-1 or PD-L1 pathways. In the context of neoadjuvant chemotherapy (NAC) for breast cancer, especially triple-negative and HER2-positive subtypes, TILs have emerged as a good predictor of pathological complete response (pCR). Denkert et al. found that increasing TIL density was substantially linked with higher pCR rates, regardless of molecular subtype, supporting their

Biomarker	Mechanism	Clinical implication	PubMed reference
Tumor mutational burden (TMB)	Somatic mutation load increases neoantigen presentation	Predicts response to immune checkpoint inhibitors (ICIs)	Chan et al. [17]
Neutrophil-to-lymphocyte ratio (NLR)	Represents the equilibrium between innate (neutrophil) and adaptive (lymphocyte) immunity.	Prognostic and predictive marker for outcomes and treatment response	Templeton et al. [22]
Programmed death-ligand 1 (PD-L1)	Expression of immunological checkpoint ligands on tumor and immune cells	Guides anti-PD-1/PD-L1 therapy selection	Garon et al. [28]
Mismatch repair deficiency (dMMR)/microsatellite instability (MSI)	Deficiency in DNA repair results in hypermutation.	Predicts benefit from ICIs, especially in colorectal and other cancers	Le et al. [34]
Tumor-infiltrating lymphocytes (TILs)	Extent of immune cell infiltration in the tumor microenvironment	Correlates with survival benefit and response to immunotherapy	Denkert et al. [9]

Table 1.
Overview of predictive biomarkers and their clinical implications.

potential as a predictive biomarker for chemotherapeutic response [9]. Furthermore, TILs are being studied as both a biomarker for immunotherapy response and a possible cellular treatment method, notably in melanoma and other highly immunogenic tumors.

3.5.1 Clinical scenario

A 40-year-old woman was diagnosed with stage IV cutaneous melanoma, with metastases to the lungs and liver. Histopathological examination of the main tumor demonstrated a substantial infiltration of lymphocytes inside the tumor parenchyma and surrounding stroma, indicative of a TIL-high phenotype. Molecular profiling revealed no actionable driver mutations, and PD-L1 expression was moderate. Given the elevated TIL density, indicative of a pre-existing anti-tumor immune response, the patient started treatment with pembrolizumab 100 mg, delivered intravenously every 3 weeks. Following a year of medication, the patient exhibited enduring clinical stability, with imaging revealing a decrease in metastatic lesion size and an absence of disease progression (**Table 1**).

4. Conclusion

Predictive biomarkers are crucial in enhancing immunotherapy by identifying individuals who are most likely to benefit and facilitating personalized treatment approaches. Despite considerable advancements, the absence of standardized diagnostic methodologies and the diversity across tumor types remain obstacles. Progress in molecular diagnostics, together with continuous clinical validation, will be essential in optimizing biomarker use and improving the accuracy, effectiveness, and cost-efficiency of cancer immunotherapy.

5. Future directions

In the last 10 years, immunotherapy has transformed the treatment paradigm in oncology, providing sustained responses in several solid and haematologic cancers with immune checkpoint inhibitors and combination approaches. Notwithstanding these gains, the clinical advantage is confined to a specific fraction of patients, highlighting the pressing want for validated predictive biomarkers to inform therapy selection, forecast therapeutic response, and mitigate avoidable toxicity and expenses. Established biomarkers such as PD-L1, TMB, and MSI-H/dMMR exhibit clinical utility; however, emerging markers—including tumor microbiome components, circulating exosomal PD-L1, soluble immune checkpoints, T-cell receptor (TCR) clonality, and gene expression profiles such as the interferon-gamma (IFN- γ) signature—demonstrate potential in preliminary studies.

Nevertheless, the majority of these new biomarkers are deficient in extensive validation and standardization. Future initiatives must focus on prospective, biomarker-enhanced randomized clinical trials to demonstrate repeatability, identify clinically significant cutoff thresholds, and guarantee cross-platform consistency. Furthermore, using composite biomarker panels that amalgamate genomic, proteomic, and immunologic data may improve prognostic accuracy compared to single-marker methodologies. Artificial intelligence and machine learning techniques

has the capability to enhance biomarker-driven models via the analysis of intricate multi-omic datasets.

Integrating biomarker discovery as a fundamental aim in clinical trial design, especially in early-phase studies, is crucial for the advancement of precision immunology. Ultimately, implementing these findings into standard practice would need extensive coordination among doctors, pathologists, regulatory agencies, and industry stakeholders to synchronize assay development and regulatory clearance. By resolving these deficiencies, further research may enhance the applicability of immunotherapy, customizing it for a wider and more heterogeneous patient demographic.

Acknowledgements

I Praise and thank God Almighty who has been my source of strength in every step of my life and foundation of my knowledge and wisdom.

I would like to express my profound gratitude to Dr. Piyush Kumar, Professor and Head, Department of Radiation Oncology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly. His exceptional leadership, expertise, and mentorship have been a constant source of inspiration for me. I am deeply thankful for his invaluable guidance, constructive suggestions, and unwavering support. Despite his demanding schedule, he has always been accessible, patiently clarifying doubts and sharing his vast knowledge. I feel immensely fortunate to have had the privilege of working under his guidance and learning from his research acumen. His influence has not only shaped my research but also inspired me to strive for excellence."

I would like to extend my heartfelt gratitude to Dr. Srinivas Naidu, Assistant Professor, Department of Medical Oncology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly. His unwavering support and encouragement during challenging times have been a beacon of hope. His guidance and care have not only helped me navigate difficult situations but have also instilled in me the confidence to persevere. I am deeply grateful for his kindness, expertise, and mentorship. Thank you for being an exceptional mentor and a source of inspiration.

Finally I must express my very profound gratitude to my parents for their unfailing emotional support and continuous encouragement throughout my years of study and through the process of researching and writing this chapter. This accomplishment would not have been possible without them.

Abbreviations

ASCO	American Society of Clinical Oncology
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
CRC	colorectal cancer
dMMR	deficient mismatch repair
DCR	disease control rate
FDA	food and drug administration
HER2	human epidermal growth factor receptor 2
ICIs	immune checkpoint inhibitors
IFN- γ	interferon-gamma
IHC	immunohistochemistry
irAEs	immune-related adverse events

Mb	megabase
MHC	major histocompatibility complex
MSI	microsatellite instability
MSI-H	microsatellite instability-high
MSS	microsatellite stable
NAC	neoadjuvant chemotherapy
NGS	next-generation sequencing
NK cells	natural killer cells
NLR	neutrophil-to-lymphocyte ratio
NSCLC	non-small cell lung cancer
ORR	objective response rate
OS	overall survival
PCR	polymerase chain reaction
PD-1	programmed cell death protein 1
PD-L1	programmed death-ligand 1
PFS	progression-free survival
pMMR	proficient mismatch repair
POLE	DNA polymerase epsilon
pCR	pathological complete response
ROS	reactive oxygen species
RCC	renal cell carcinoma
SNP	single nucleotide polymorphism
SOFA	sequential organ failure assessment
TCR	T-cell receptor
TILs	tumor-infiltrating lymphocytes
TMB	tumor mutational burden
TME	tumor microenvironment
TPS	tumor proportion score

Author details

Laya K. Sathyan^{1*}, Srinivas Naidu¹ and Piyush Kumar²

1 Medical Oncology, SRMSIMS, Bareilly, Uttar Pradesh, India

2 Radiation Oncology, SRMSIMS, Bareilly, Uttar Pradesh, India

*Address all correspondence to: layaksreelayam@gmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Dizon DS, Krilov L, Cohen E, et al. Clinical cancer advances 2016: Annual report on progress against cancer from the American Society of Clinical Oncology. *Journal of Clinical Oncology*. 2016;**34**:987-1011
- [2] Sharma P, Allison JP. The future of immune checkpoint therapy. *Science*. 2015;**348**(6230):56-61
- [3] Gettinger SN, Horn L, Gandhi L, et al. Overall survival and long-term safety of nivolumab (anti-programmed death 1 antibody, BMS-936558, ONO-4538) in patients with previously treated advanced non-small-cell lung cancer. *Journal of Clinical Oncology*. 2015;**33**:2004-2012
- [4] Larkin J, Lao CD, Urba WJ, et al. Efficacy and safety of nivolumab in patients with BRAF V600 mutant and BRAF wild-type advanced melanoma: A pooled analysis of 4 clinical trials. *JAMA Oncology*. 2015;**1**:433-440
- [5] Motzer RJ, Rini BI, McDermott DF, Redman BG, Kuzel TM, Harrison MR, et al. Nivolumab for metastatic renal cell carcinoma: Results of a randomized phase II trial. *Journal of Clinical Oncology*. 2015;**33**(13):1430-1437
- [6] Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *The New England Journal of Medicine*. 2015;**372**(21):2006-2017
- [7] Sha D, Jin Z, Budczies J, Kluck K, Stenzinger A, Sinicrope FA. Tumor mutational burden as a predictive biomarker in solid tumors. *Cancer Discovery*. 2020;**10**(12):1808-1825
- [8] Pajens ST, Vledder A, de Bruyn M, Nijman HW. Tumor-infiltrating lymphocytes in the immunotherapy era. *Cellular & Molecular Immunology*. 2021;**18**(4):842-859
- [9] Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: A pooled analysis of 3771 patients treated with neoadjuvant therapy. *The Lancet Oncology*. 2018;**19**(1):40-50
- [10] Strimbu K, Tavel JA. What are biomarkers? *Current Opinion in HIV and AIDS*. 2010;**5**(6):463-466
- [11] Poste G. Bring on the biomarkers. *Nature*. 2011;**469**(7329):156-157
- [12] Baselga J, Cortés J, Kim SB, Im SA, Hegg R, Im YH, et al. Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. *The New England Journal of Medicine*. 2012;**366**(2):109-119
- [13] Wang DY, Salem JE, Cohen JV, Chandra S, Menzer C, Ye F, et al. Fatal toxic effects associated with immune checkpoint inhibitors: A systematic review and meta-analysis. *JAMA Oncology*. 2018;**4**(12):1721-1728
- [14] Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: Recent progress and potential biomarkers. *Experimental & Molecular Medicine*. 2018;**50**(12):1-11
- [15] Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nature Reviews. Clinical Oncology*. 2017;**14**(12):717-734

- [16] Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*. 2004;**21**(2):137-148
- [17] Chan TA, Yarchoan M, Jaffee E, Swanton C, Quezada SA, Stenzinger A, et al. Development of tumor mutation burden as an immunotherapy biomarker: Utility for the oncology clinic. *Annals of Oncology*. 2019;**30**(1):44-56
- [18] Govindan R, Ding L, Griffith M, Subramanian J, Dees ND, Kanchi KL, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. *Cell*. 2012;**150**(6):1121-1134
- [19] Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. Socinski MA; CheckMate 026 Investigators. First-line Nivolumab in stage IV or recurrent non-small-cell lung cancer. *The New England Journal of Medicine*. 2017;**376**(25):2415-2426
- [20] Forget P, Khalifa C, Defour JP, Latinne D, Van Pel MC, De Kock M. What is the normal value of the neutrophil-to-lymphocyte ratio? *BMC Research Notes*. 2017;**10**(1):12
- [21] Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell*. 2010;**140**(6):883-899
- [22] Templeton AJ, McNamara MG, Šeruga B, Vera-Badillo FE, Aneja P, Ocaña A, et al. Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: A systematic review and meta-analysis. *Journal of the National Cancer Institute*. 2014;**106**(6):dju124
- [23] Diem S, Schmid S, Krapf M, Flatz L, Born D, Jochum W, et al. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. *Lung Cancer*. 2017;**111**:176-181
- [24] Huang Z, Fu Z, Huang W, Huang K. Prognostic value of neutrophil-to-lymphocyte ratio in sepsis: A meta-analysis. *The American Journal of Emergency Medicine*. 2020;**38**(3):641-647
- [25] Drăgoescu AN, Pădureanu V, Stănculescu AD, Chiuțu LC, Tomescu P, Geormăneanu C, et al. Neutrophil to lymphocyte ratio (NLR)-a useful tool for the prognosis of sepsis in the ICU. *Biomedicine*. 2021;**10**(1):75
- [26] Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nature Medicine*. 2002;**8**(8):793-800
- [27] Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: A common denominator approach to cancer therapy. *Cancer Cell*. 2015;**27**(4):450-461
- [28] Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *The New England Journal of Medicine*. 2015;**372**(21):2018-2028
- [29] Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): A phase 3, open-label, multicentre randomised controlled trial. *Lancet*. 2017;**389**(10066):255-265
- [30] Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell

lung cancer. *The New England Journal of Medicine*. 2015;**373**(2):123-135

[31] Hause RJ, Pritchard CC, Shendure J, Salipante SJ. Classification and characterization of microsatellite instability across 18 cancer types. *Nature Medicine*. 2016;**22**(11):1342-1350. DOI: 10.1038/nm.4191. Epub 2016 Oct 3. Erratum in: *Nat Med*. 2017;**23**(10):1241

[32] Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. *Nature Reviews. Clinical Oncology*. 2010;**7**(3):153-162

[33] Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology*. 2010;**138**(6):2073-2087.e3

[34] Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *The New England Journal of Medicine*. 2015;**372**(26):2509-2520

[35] Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): An open-label, multicentre, phase 2 study. *The Lancet Oncology*. 2017;**18**(9):1182-1191

[36] Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: Impact on clinical outcome. *Nature Reviews. Cancer*. 2012;**12**(4):298-306

Section 2

Navigating the Tumor Landscape: Immune Evasion and Interactions

The Science of PD-1/PD-L1 Pathway in Gliomas: Far beyond the Obvious

Thamiris Becker Scheffel and Karine Rech Begnini

Abstract

Gliomas are extremely aggressive and challenging to treat, with their resistance often linked to the pronounced immunosuppression within the tumor microenvironment. Influenced by hypoxia, cytokines, and oncogenic signaling, tumor cells exhibit aberrant expression of programmed cell death ligand-1 (PD-L1), positioning the PD-1/PD-L1 pathway as a pivotal mechanism among immune checkpoints in promoting immune evasion. Despite significant progress in immunotherapy for other cancers, approaches targeting the PD-1/PD-L1 axis continue to face substantial barriers in gliomas. Resistance to immune checkpoint inhibitors has become a frequent clinical challenge, underscoring the urgent need for a deeper understanding of glioma immunobiology. Notably, beyond its extracellular immune-regulatory role, PD-L1 also exerts intracellular signaling functions in the cytosol and nucleus of cancer cells, contributing to stress response regulation and tumor survival. Given the diverse mechanisms driving tumor initiation and progression, this chapter aims to unravel the multifaceted roles of the PD-1/PD-L1 pathway and the PD-L1 expression, with a particular focus on its direct effects on glioma cells.

Keywords: immunosuppression, programmed cell death ligand-1 (PD-L1), glioblastoma, immunotherapy, intracellular PD-L1

1. Introduction

The scenery of the immune checkpoints in gliomas reads like a paradox within cancer immunology, a story of high hopes met by persistent complexity. From the moment PD-1/PD-L1 emerged as a pivotal immune checkpoint, the scientific community anticipated a revolution: a way to overcome the immunosuppression that gliomas impose within the brain. However, gliomas consistently evade therapeutic approaches.

Gliomas remain among the most aggressive and therapeutically challenging brain tumors, characterized by their highly immunosuppressive tumor microenvironment (TME) and remarkable resistance to conventional and novel therapies [1, 2]. The programmed cell death protein 1 (PD-1) and its ligand PD-L1 axis

have emerged as critical immune checkpoints mediating tumor immune evasion across various cancers [3, 4]. However, unlike in melanoma or non-small cell lung cancer, immune checkpoint inhibitors targeting PD-1/PD-L1 have demonstrated limited efficacy in gliomas [5, 6].

This discrepancy underscores the complex immunobiology of gliomas, where factors such as hypoxia, oncogenic signaling pathways, and a highly suppressive microenvironment drive aberrant PD-L1 expression and contribute to immune escape [7]. Notably, emerging evidence reveals that PD-L1's role spreads beyond its canonical extracellular immune-regulatory function. Intracellular PD-L1 localizes to the cytoplasm and nucleus, modulating tumor-intrinsic signaling pathways involved in stress response, cell survival, and therapy resistance [8, 9].

This chapter aims to unravel the complex biology of the PD-1/PD-L1 axis in gliomas, integrating classical immunological concepts with emerging insights into tumor-intrinsic roles, thereby guiding future research and clinical strategies beyond the obvious.

2. The glioma microenvironment: A haven for immune evasion

The tumor microenvironment in gliomas is formed by a heterogeneous composition of cell types such as astrocytes and stromal cells, endothelial cells, associated fibroblasts, glioma stem cells, and a complex range of infiltrating immune cells [10].

Three main microenvironments have been described in GBM: the perivascular niche, the invasive front, and the hypoxic-necrotic core (**Figure 1**).

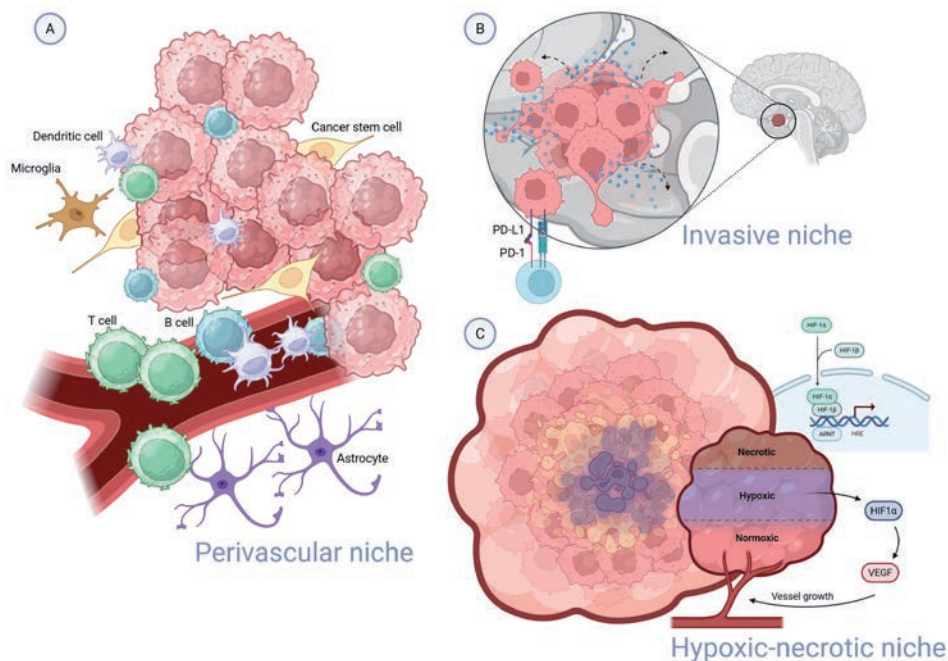


Figure 1.

Tumor niches in gliomas. (A) Perivascular niche, providing vascular support and cellular protection; (B) invasive niche, where tumor cells migrate and infiltrate surrounding tissue; (C) hypoxic-necrotic niche, characterized by adaptation and resistance in low-oxygen, necrotic regions. This figure was created with BioRender.com.

Each microenvironment acts as a unique modulator, activating a series of cellular pathways, which in turn serve as key components in the dynamism of the TME. The perivascular niche is composed of both neoplastic and non-neoplastic cells, including endothelial cells, immune cells, reactive astrocytes, neural progenitor cells, and tumor stem cells [11, 12].

Astrocytes are the main components of the brain stroma and have been strongly linked to the malignant phenotype of gliomas, playing important roles in chemotherapy resistance [13, 14]. Under physiological conditions, astrocytes are dynamic in performing their functions, mainly involved in the rigidity and structure of the blood-brain barrier (BBB), modulating synaptic activity, and in brain injury repair through astrogliosis [14]. Astrogliosis is characterized by aberrant astrocyte proliferation, an increase in the number of intermediate filaments, and cellular hypertrophy. It can occur in response to gliomagenesis, as tumor development causes damage and invasion of the adjacent tissue. In this process, while the goal is tissue repair and reconstruction, under the influence of GBM cells, reactive astrocytes may support tumor growth and make tumor cells resistant to therapy [15]. According to Chen et al., co-culture of GBM cells with astrocytes significantly decreased chemotherapy-induced apoptosis [16].

The perivascular niche created by GBM also results in changes in endothelial cells, which exhibit higher expression of adhesion molecules and loosening of cell junctions, making the BBB more permeable. This change in brain permeability facilitates leukocyte extravasation and immune infiltration of the tumor mass [17].

Particularly, the arising significance of immune infiltrate and its products in the process of tumor malignancy has been demonstrated. The immunobiology of gliomas involves a complex interplay between tumor cells and the immune microenvironment and has been directly linked to the processes of survival, proliferation, and tumor migration [10, 18]. Innate immune cells (macrophages, mast cells, neutrophils, dendritic cells, myeloid-derived suppressor cells (MDSCs), and natural killer cells) and adaptive immune cells (T and B lymphocytes) directly interact with neoplastic cells, shaping tumor behavior. It is important to emphasize that immune cells can either support or hinder therapeutic efficacy, and their levels of activation and location within the TME can also vary [17, 19].

Tumor associated macrophages (TAMs) and microglia are the dominant immune populations in GBMs, comprising about 30–40% of the cells in the tumor microenvironment [17, 20]. Both cell types play immune roles that includes migration to the injury site, phagocytosis, antigen presentation, and release of cytokines and chemokines [21]. Within the tumor, under the influence of hypoxia and chemokines, both macrophages and microglia can polarize into distinct phenotypes: M1 phenotype indicates inflammatory and anti-tumor macrophages, while M2 phenotype is associated with the tumor (TAMs), having anti-inflammatory and pro-tumoral characteristics, and have been linked to neovascularization formation and, consequently, to tumor migration and invasion potentials [21, 22]. However, macrophages and microglia are mononuclear cells characterized by considerable plasticity, and the classical notion that macrophages are polarized and present a few differentiated states in TME has been questioned by single cell analysis [23, 24]. Evidence suggests that antitumoral and pro-tumoral phenotypes are the opposite ends of a continuum spectrum with diverse cell states co-existing in a graded scale of phenotypes [24, 25]. In glioma samples, macrophage population found in the TME have mixed expression of both M1 and M2 markers in the same cells [26], highlighting a dynamic behavior of these cells over time and course of disease progression.

Diffuse gliomas are so named due to their high capacity to invade the surrounding tissue. Although primary brain tumors generally do not metastasize, malignant gliomas are characterized by significant invasion of adjacent brain tissue, leading to the death of parenchymal cells and causing tissue toxicity [27, 28]. The cells of the invasive niche of GBM can infiltrate individually, migrate along blood vessels, or spread through the invasive edge, which represents a critical third niche of the glioma microenvironment [29]. The perivascular space, the interstitial spaces of the parenchyma, and the invasive edge contain a dense composition of extracellular matrix molecules, primarily produced by astrocytes and oligodendrocytes [30]. Astrocytes, microglia, and endothelial cells also express proteases that promote extracellular matrix remodeling while simultaneously regulating the release of growth factors and chemokines, facilitating tumor invasion [31].

Pseudopalisading necrosis and microvascular hyperplasia are hallmark histopathological features of GBM [32]. This strongly supports the functional involvement of the hypoxic-necrotic niche in tumor progression and aggressiveness [11]. Studies have identified hypoxia as a crucial regulator of survival pathways and immune evasion in GBM cells [33–35].

The necrotic area at the center of the hypoxic-necrotic niche releases pro-inflammatory signals capable of reshaping the immune response within the tumor microenvironment. In pseudopalisading regions, hypoxia-inducible factors (HIFs) are upregulated and drive the expression of mediators such as vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8), which are involved in various aspects of cellular homeostasis, including survival, invasion, and angiogenesis [35–38].

Overall, the glioma microenvironment is highly immunosuppressive, with a strong tendency to promote tumor growth [20].

2.1 Unmasking immunosuppression in gliomas

The close relationship between inflammation and cancer was first proposed by Rudolf Virchow when the prevalence of leukocytes in tumors was observed. Since then, the connection between tumors and the immune system has been implicated in cancer prevention and treatment. Currently, one of the main therapeutic challenges in GBM is blocking the tumor-related mechanisms of immunosuppression (**Figure 2**) [39–41].

Specifically, GBM exhibits a complex relationship among immune surveillance, immunosuppression, and gliomagenesis.

Indeed, GBM is considered an immunologically “cold” tumor (**Figure 3**). The brain has always been described as an organ with a low immune response, due to the presence of the highly selective BBB, as well as the low expression of the major histocompatibility complex (MHC) [42]. Furthermore, studies show that patients with GBM present decreased levels of circulating T lymphocytes, which makes them more susceptible to viral infections and impairs their tumor cytotoxic potential [43]. Overall, the conditions of the TME determine the balance between pro-tumor inflammation and anti-tumor responses, and in the case of GBM, several anti-inflammatory mechanisms are implicated in recurrence and poor prognosis [44].

The TME exhibits various cellular communication pathways. These include direct cellular interactions through membrane receptors and their ligands; the release of soluble factors such as cytokines, chemokines, and metabolites; and extracellular vesicles (EVs), which can carry numerous bioactive molecules responsible for altering the expression of tumor-promoting and suppressing genes in receptor cells [45].

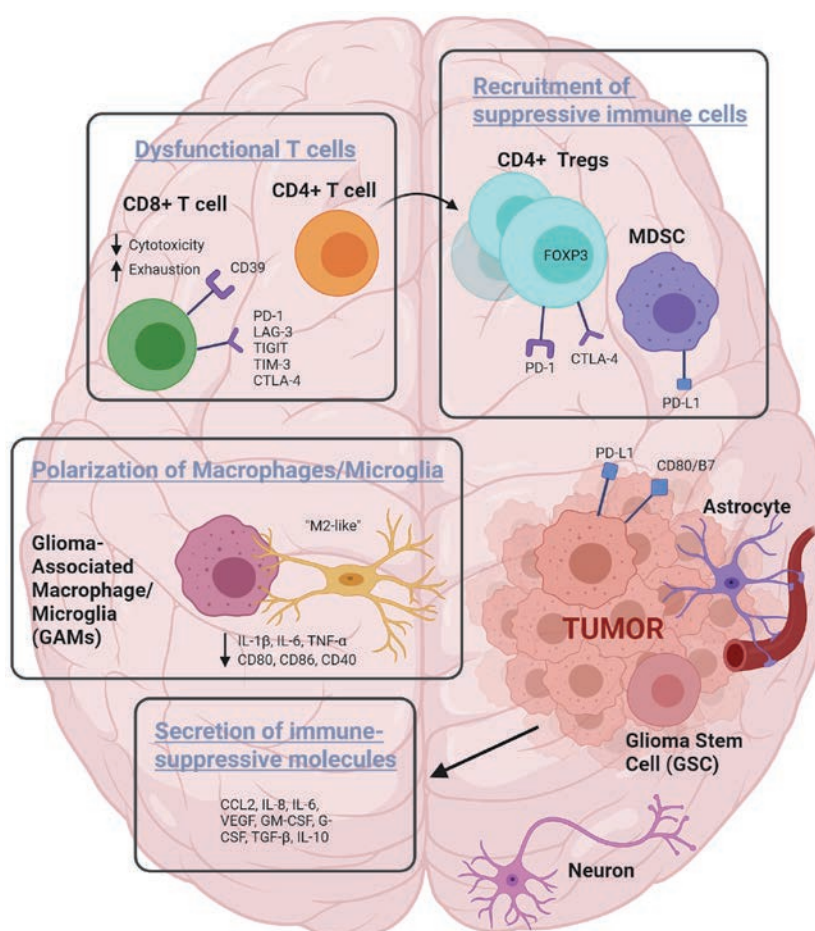


Figure 2.
 Immunosuppressive GBM microenvironment. This figure was reproduced from BioRender.com.

One of the main effects of cellular signaling within the TME is the recruitment of immune cells. The immune infiltrate in the tumor is primarily composed of lymphocytes, macrophages, and microglia [44]. These cells present an immunosuppressive phenotype induced by GBM cells, capable of modulating the production of growth factors, angiogenic signals, and those inducing tumor migration [39, 46, 47].

Chemokines are generally important in regulating responses mediated by CD4+ and CD8+ T lymphocytes. M1 macrophages typically produce Th1-type responses, whereas M2 macrophages, which prevail in the TME, initiate Th2-type responses [48]. These responses are linked to the different cytokine profiles released by T lymphocyte subtypes. Th1 cytokines (pro-inflammatory), such as interferon- γ (IFN- γ), IL-2, IL-12, and tumor necrosis factor- α (TNF- α), promote cell-mediated immune responses and therefore have potential anti-tumor effects. In contrast, Th2 cytokines (immunosuppressive), such as IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, stimulate humoral immune responses and negatively regulate specific immune responses. Th3 cytokines, such as TGF- β , are also strongly immunosuppressive [43].

Multiple cell types can secrete TGF- β , which is elevated in the serum of patients with GBM and has been implicated as one of the main factors inducing

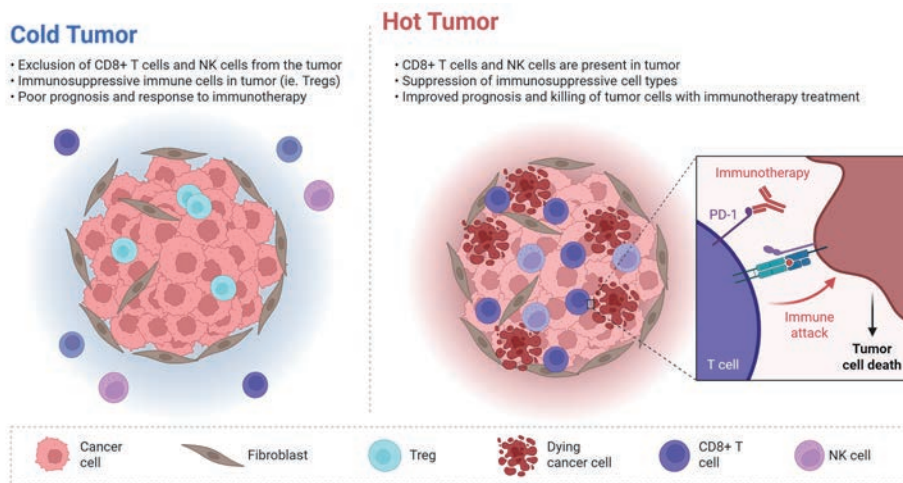


Figure 3. “Cold” and “Hot” tumors. “Cold” tumors exhibit poor T cell infiltration and low inflammation; “Hot” tumors are immunologically active, with robust immune cell infiltration, high inflammation, and potential responsiveness to immunotherapy. This figure was reproduced from BioRender.com.

regulatory T lymphocytes (Tregs) [49, 50]. Tregs are a subset of CD4+ T cells that express the transcription factor Foxp3. Indeed, it is well established that TGF- β signaling in the presence of IL-2 can induce Foxp3 expression in naive T cells [51, 52]. Furthermore, Francisco et al. [53] demonstrated that PD-L1 could also induce Tregs in vitro. According to the authors, PD-L1 increased Foxp3 expression and immunosuppressive capacity in Tregs. Additionally, it was reported that PD-L1 could convert naive CD4+ cells into Tregs through simultaneous negative regulation of Akt/mTOR/ERK and positive regulation of PTEN. Therefore, the PD-1/PD-L1 immune checkpoint axis also appears to play a crucial role in the development and function of Tregs [53].

Tregs are divided into two groups based on their origin: natural or peripherally induced. As the name suggests, natural Tregs (nTregs) develop in the thymus and are mitotically quiescent under basal conditions, requiring antigenic stimulation to perform their functions in maintaining peripheral tolerance. Alternatively, peripherally induced Tregs (iTregs) arise when co-exposed to antigen-presenting cells (APCs) and high levels of TGF- β or T cell receptor stimulation [54]. It is important to note that natural Tregs are prevalent in brain tumors [55].

Tregs exhibit immunosuppressive abilities through several mechanisms. Specifically, (a) Tregs consume IL-2 from the TME to suppress the activation and proliferation of effector T cells; (b) express CTLA-4, reducing the stimulatory signal from APCs to CD4+ and CD8+ T cells; (c) secrete Th2 and Th3 cytokines, impairing the anti-tumor effects mediated by innate immunity; (d) hydrolyze the immune-stimulatory molecule ATP and activate the adenosine pathway, contributing to a potent immunosuppressive environment; and finally, (e) induce direct death of T cells or APCs by producing granzymes and perforin [56, 57].

The increased presence of Tregs in the TME and the negative regulation of effector T cells are associated with poor prognosis in several cancer types [57]. The implication of these cells in glioma aggressiveness has also been suggested by the positive correlation between their clonal levels and tumor malignancy grade [55, 58].

Additionally, the interaction between the immune system and tumor stem cells has been demonstrated. In vitro studies have shown that these cells can suppress cytotoxic responses, induce and modulate macrophage functions, and secrete factors such as IL-10 and TGF- β , strongly suggesting an important role of tumor stem cells in maintaining an immunosuppressive microenvironment at the tumor site [59].

3. The PD-1/PD-L1 pathway: Canonical functions

The PD-1/PD-L1 pathway is essential in regulating the immune system, influencing both tumor progression and immune response. The transmembrane receptor PD-1 (CD279), encoded by the *PDCD1* gene, belongs to the CD28 family of immunoglobulins. Its expression is induced in activated T and B lymphocytes, natural killer cells, dendritic cells, and monocytes [4]. The protein structure includes an extracellular domain, a transmembrane region, and a cytoplasmic tail. Activation of PD-1 leads to the phosphorylation of tyrosine residues within the cytoplasmic tail at high-affinity sites for SH2-domain-containing phosphatases (SHP-2 and SHP-1), resulting in increased PTEN activity and attenuation of the PI3K and Akt pathways [60].

PD-1 is commonly activated by its ligand PD-L1 (B7-H1; CD274), which is constitutively expressed in myeloid cells and can be induced in B lymphocytes and parenchymal cells following exposure to pro-inflammatory stimuli, or by PD-L2 (B7-DC; CD273), which has a more restricted expression pattern [61, 62]. Physiologically, the PD-1/PD-L1 pathway plays a critical role in controlling prolonged inflammatory responses, often preventing autoimmunity and maintaining immune homeostasis [63].

Under the influence of hypoxia, cytokines, and oncogenes, PD-L1 and PD-L2 are aberrantly expressed by tumor cells, making the PD-1/PD-L1 axis one of the key mechanisms responsible for tumor immune evasion [64, 65]. It has been demonstrated that PD-L1/PD-L2 expressed on tumor cells binds to PD-1 on T lymphocytes, playing a critical role in tumor initiation and progression. This interaction induces exhaustion of effector T lymphocytes, neutralizing antitumor signaling and promoting Treg activation [66–68].

In this context, PD-L1 expression is regulated through multiple mechanisms, including genetic alterations, dysregulated oncogenic signaling pathways, post-transcriptional and post-translational modifications, influences from the TME, cytotoxic therapies, and immune activation. PD-L1 expression could be activated by pro-inflammatory signals. IFN γ triggers the JAK-STAT pathway, leading to the activation of the interferon-responsible factor 1 (IRF1), which then binds to the PD-L1 promoter [69]. TNF α and IFN γ also activate the NF- κ B pathway, further increasing PD-L1 expression. These mechanisms explain why PD-L1 is highly expressed in inflamed tissues and immune-infiltrated tumors (“hot” tumors) [70]. However, regulation varies depending on the cell type and conditions. In GBM cells, PD-L1 expression has been associated with tumor invasiveness and aggressiveness [7, 71–74]. Studies have highlighted heterogeneity in PD-L1 expression, with higher levels observed at the invasive edge compared to the tumor core. This localization facilitates glioma mobility along adjacent tissue [75].

Oncogenic pathways play a crucial role in promoting PD-L1 expression, extending beyond their contribution to cancer progression. Certain oncogenic transcription factors, such as MYC, HIF-1 α , and HIF-2 α , directly regulate PD-L1 transcription by binding to its promoter. Additionally, activating mutations in KRAS, BRAF, and

EGFR drive PD-L1 expression through the MEK-ERK pathway, in conjunction with STAT1 activation [76]. Studies have also shown that PD-L1 expression in gliomas is regulated by STAT3 [77].

Many cancer cells constitutively express high levels of PD-L1 due to oncogenic pathways such as Ras, Akt-mTOR, EGFR, MEK-ERK, and MAPK p38 [62]. This helps tumors to resist immune attacks in inflammatory environments. Additionally, oncogenic PTEN mutations have been shown to upregulate PD-L1 expression in gliomas [77].

Other factors, such as hypoxia and epigenetic changes, also influence PD-L1 expression [78]. For instance, certain microRNAs (miR-513 and miR-152) reduce PD-L1 translation, while some cancer cells modify the 3'UTR of PD-L1 mRNA to keep its expression high [79–81].

PD-L1 stability is regulated by its interactions with membrane proteins such as CMTM6 and CMTM4, which prevent its degradation and enhance its expression on the cell surface [82, 83]. CMTM6, a type 3 transmembrane protein, associates with PD-L1 on the plasma membrane and within recycling endosomes. By inhibiting PD-L1 ubiquitination and degradation, it extends PD-L1's half-life, reinforcing its function in immune regulation [82].

Notably, Tregs have emerged as critical targets in cancer therapy, as they express both PD-1 and PD-L1 [55]. The inhibitory activity of Tregs on effector T lymphocytes typically occurs through cell-to-cell contact, partially mediated by CTLA-4 or PD-1/PD-L1 interactions [63]. Bedke et al. demonstrated a significant increase in the immunosuppressive activity of CD4 + Foxp3+ T lymphocytes mediated by the upregulation of PD-1 expression after contact with endothelial cells during blood extravasation into the inflamed tissue [84]. Additionally, in vitro studies have shown that the PD-1/PD-L1 pathway may reduce T cell adhesion capacity and impair immune synapse formation [85].

In addition to these regulatory mechanisms, the Hippo pathway plays a key role in cancer development and progression, mainly through the dysregulation of its transducers, TAZ and YAP. These proteins regulate PD-L1 expression in tumor cells, influencing immune-related genes involved in inflammasome formation, and complement activation [86].

Thus, immune checkpoint inhibition, particularly targeting the PD-1/PD-L1 axis, has emerged as a promising strategy in cancer therapy (**Figure 4**). Pharmacological blockade can restore the number of effector T lymphocytes and promote cytotoxic immunity against chemotherapy-resistant tumors. Furthermore, this therapy can stimulate the production of pro-inflammatory cytokines, reestablishing anti-tumor immunity [87]. The efficacy of PD-1/PD-L1 blockade in tumor eradication has been extensively investigated. In recent years, immunotherapy has demonstrated significant clinical benefits in the treatment of various cancer types, offering durable responses and reduced toxicity compared to chemotherapy [88].

Nivolumab was the first IgG4 monoclonal antibody targeting the PD-1 receptor to show clinically relevant activity in unresectable or metastatic melanoma, advanced or metastatic non-small cell lung carcinoma, and metastatic renal cell carcinoma [89, 90].

Clinical phase studies are being conducted to evaluate the efficacy of these monoclonal antibodies in the treatment of gliomas [91]. Although highly effective, anti-PD1/PD-L1 therapies have not shown significant benefits for many GBM patients, resulting in little to no effect on overall survival rates compared to existing therapies [91, 92]. Therefore, new studies involving combination therapies may be promising strategies to enhance the response to immunotherapy [93].

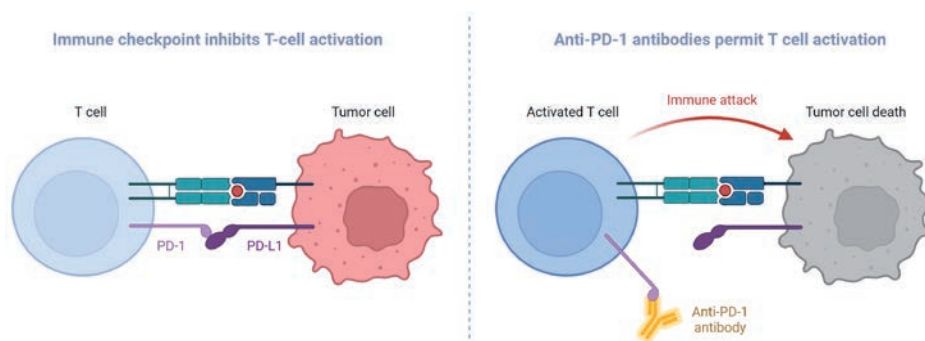


Figure 4.
 Immune checkpoint inhibition on tumor cells. Anti-PD-1/PD-L1 antibodies can restore the effector T lymphocytes immunity and promote cytotoxicity against chemotherapy-resistant tumors. This figure was reproduced from BioRender.com.

Interestingly, PD-L1 has been observed to undergo internalization from the cell surface within 30 minutes at 37°C after treatment with anti-PD-L1 antibodies. This process leads to the cessation of downstream signaling events, including mTOR and Akt activity, as well as the expression of glycolytic enzymes [94]. Such findings further underscore the relevance of PD-L1 as an intracellular signaling molecule beyond its well-established immune functions.

4. Intracellular PD-L1: The hidden functions beyond immunity

The direct immune role of PD-L1 has been extensively studied; however, its subcellular and extracellular signaling network within cancer cells remains largely unexplored.

PD-L1 is a type I transmembrane glycoprotein belonging to the immunoglobulin (Ig) family. The protein structure of PD-L1 includes an extracellular domain composed of a signal peptide, an Ig-like V-type domain and two Ig-like C-type, a transmembrane region, and a cytoplasmic tail (**Figure 5**) [95].

PD-L1 is synthesized in the endoplasmic reticulum (ER) and modified in the Golgi complex (**Figure 5**). From there, it can follow different paths: remain in the cytoplasm (cPD-L1), translocate to the nucleus (nPD-L1), be secreted into the extracellular space (sPD-L1), or be transported to the cell membrane (mPD-L1) [96–98]. The cytoplasmic and nuclear forms, collectively termed intracellular PD-L1, exhibit functions distinct from its classical immune checkpoint role at the membrane [96]. Membrane-bound PD-L1 can also be internalized via endocytosis, leading to the release of PD-L1-containing exosomes (exoPD-L1), or form microvesicles through direct membrane budding (mvPD-L1) [98]. In short, PD-L1 exists in a highly dynamic state. Understanding subcellular and extracellular roles of PD-L1 might shed light on the resistance to anti-PD1/PDL1 therapy and represent an opportunity for targeted therapies that disrupt PD-L1's functions beyond its interaction with PD-1.

Non-canonical forms of PD-L1, mainly cPD-L1, nPD-L1, and exoPD-L1, are functionally distinct and play critical roles in regulating tumor cell proliferation, modulating immune responses, orchestrating the DNA damage response, and influencing gene expression [99–103].

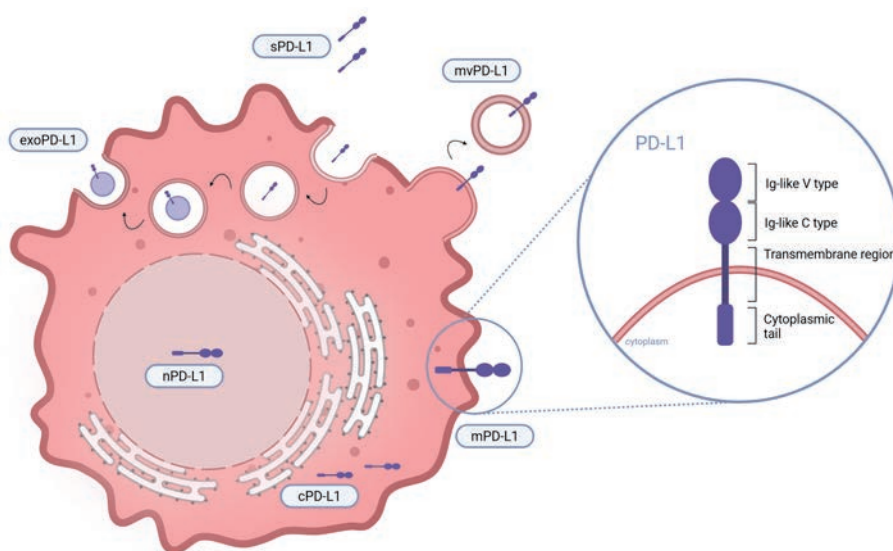


Figure 5.

Subcellular and extracellular dynamic state of PD-L1. PD-L1 is synthesized in the endoplasmic reticulum, modified in the Golgi complex, and exists as intracellular [cytoplasmic (cPD-L1), nuclear (nPD-L1)], membrane-bound (mPD-L1), or extracellular forms: soluble (sPD-L1), exosomal (exoPD-L1), and microvesicle-associated (mvPD-L1). This figure was created with BioRender.com.

Interestingly, a study revealed that cells expressing PD-L1 lacking a functional intracellular domain lost their ability to inactivate T cells. Even when T cells expressed a signal-null PD-1, cancer cells still exhibited resistance to cytotoxicity, further reinforcing the notion that PD-L1 on cancer cells plays a direct role in mediating resistance to T cell-induced death [9].

The PD-L1 signalosome has been linked to cell survival, stress responses, and resistance to pro-apoptotic signals. Emerging evidence indicates that PD-L1 influences tumor progression through metabolic modulation, particularly in glucose metabolism. Antibodies targeting CTLA-4, PD-1, or PD-L1 have been shown to restore glucose levels within the TME in a murine sarcoma model, suggesting that these immune checkpoint inhibitors regulate cancer cell metabolism. mPD-L1 blockade in cancer cells, mediated by antibodies, can inhibit the Akt/mTOR signaling pathway, leading to a reduction in the translation of mRNAs encoding glycolytic enzymes [104].

Further supporting this metabolic role, a transcriptomic analysis of PD-L1-silenced ovarian and melanoma cells identified differentially regulated genes associated with mTOR signaling and autophagy [105]. In the absence of PD-1 engagement, mTOR and stress-associated kinases likely form a stable macromolecular complex with PD-L1. Some of these kinases are physically associated with the RMLDVEKC or DTSSK motifs, either directly or via adaptor proteins, further supporting PD-L1's role in intracellular signaling [9]. In a GBM model, upregulated PD-L1 at the invasive front binds to Akt's non-PH domain, promoting its translocation to the plasma membrane and full activation. This suppresses autophagy and enhances F-actin formation, facilitating GBM invasion under starvation stress [106]. Additionally, the loss of PTEN, a tumor suppressor and negative regulator of PI3K, strongly enhances PD-L1 expression by activating the PI3K-Akt-mTOR axis [107].

The relationship between PD-L1 and stemness has been established. In breast cancer, the absence of PD-L1 inhibits Akt and p-S6 phosphorylation, disrupting the PI3K/Akt/mTOR pathway and preventing Oct-4A phosphorylation. This indicates that PD-L1 regulates Oct-4A function through this pathway [108].

Furthermore, studies have demonstrated that high neuronal PD-L1 expression near tumor cells may serve as an immune-independent anti-tumoral mechanism. Neuronal PD-L1 induces caspase-dependent apoptosis of glioma cells [109]. Additionally, cancer cells expressing PD-L1 mediated T cell apoptosis in vitro, independently of PD-1 engagement [110]. This suggests that PD-L1 plays a role beyond its classical function of negative signaling to T cells via PD-1.

4.1 Nuclear PD-L1 (nPD-L1)

When localized in the nucleus, PD-L1 exhibits functions distinct from its well-known role as an immune checkpoint at the plasma membrane. Emerging research has demonstrated that nPD-L1 contributes to tumor-intrinsic processes, promoting cancer progression and therapy resistance [111, 112]. Although initially considered an artifact of immunofluorescence microscopy [113], it is now increasingly evident that PD-L1 translocation to the nucleus is a regulated, cell-controlled process. At the plasma membrane, PD-L1 is typically present in an acetylated form, and it was traditionally believed that nPD-L1 originated through the endocytosis of this membrane-bound PD-L1. Histone deacetylase 2 (HDAC2) and the acetyltransferase p300 interact with PD-L1 at both the cell surface and within the cytosol, mediating its deacetylation and acetylation, respectively [100]. Deacetylated mPD-L1 subsequently interacts with Huntingtin Interacting Protein 1-related Protein (HIP1R), triggering its endocytosis [114]. Once internalized, this non-acetylated form of PD-L1 is trafficked along the cytoskeleton and interacts with vimentin, facilitating its subsequent nuclear import via the importin- α/β pathway [100, 114]. Additionally, PD-L1 can translocate to the nucleus through its interaction with Karyopherin β 1 (KPNB1), a major nuclear transport receptor responsible for shuttling proteins from the cytoplasm into the nucleus (**Figure 6**) [99]. However, the precise molecular mechanisms underlying the interaction between PD-L1 and KPNB1 remain to be fully elucidated.

Alternatively, nPD-L1 may originate through post-translational modifications occurring in the ER and/or Golgi apparatus, as suggested by evidence indicating that PD-L1 subcellular localization is primarily determined by its glycosylation or deglycosylation status [96]. A hyper-N-glycosylated form of nPD-L1 has been identified in breast and colon cancer cells; this form is significantly larger than PD-L1 proteins found in the cytosol or at the plasma membrane [115]. In such cases, following translation, PD-L1 undergoes extensive glycosylation and is subsequently shuttled directly into the nucleus through interactions with glycan-binding proteins [115].

Moreover, PD-L1 localization, whether retained at the plasma membrane or translocated to subcellular compartments, is influenced by the specific positioning of its glycosylation sites [116, 117]. For instance, treatment with metformin induces aberrant PD-L1 glycosylation, leading to its sequestration in the ER, where it accumulates and is ultimately degraded via the ER-associated protein degradation (ERAD) pathway [117]. Conversely, glycosylation at the N219 residue is critical for PD-L1 membrane localization, while deglycosylation at this site facilitates its redistribution to the cytoplasm and nucleus [118].

In cancer cells, the nuclear import of PD-L1 is induced by various stressors, including hypoxia, the absence of epidermal growth factor (EGF), and treatment with

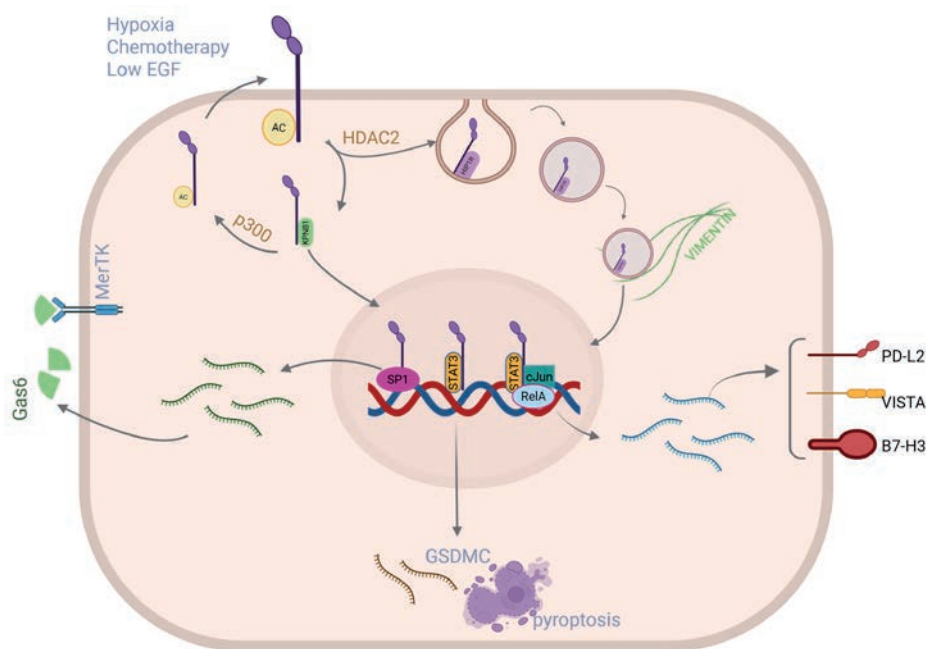


Figure 6.

PD-L1 is translocated to the nucleus where it interacts with transcription factors and chromatin-modifying complexes to regulate gene expression. This figure was created with BioRender.com.

radiation or chemotherapeutic agents [98, 114, 117, 119, 120]. Under hypoxic conditions, phosphorylated STAT3 (p-STAT3) physically interacts with PD-L1, facilitating its nuclear translocation via the importin α/β pathway [114]. Conversely, the presence of EGF promotes PD-L1 retention at the plasma membrane. Upon binding to EGFR, EGF stimulates the phosphorylation of the acetyltransferase p300 through the PI3K/Akt signaling pathway, enhancing PD-L1 acetylation and thereby preventing its nuclear translocation [119].

Chemotherapy-induced nuclear translocation of PD-L1 has been observed following treatment with various chemotherapeutic agents, including mitotic inhibitors, antibiotics, topoisomerase I and II inhibitors, antimetabolites, and alkylating agents [98]. For example, doxorubicin reduces mPD-L1 levels while increasing nPD-L1, an effect mediated by the phosphorylation of nuclear Akt. Notably, doxorubicin-induced nPD-L1 accumulation has also been detected in heart tissues, indicating that nPD-L1 translocation is not tissue-specific [120]. Radiation further promotes nPD-L1 import by enhancing its interaction with NGLY1, a de-N-glycosylating enzyme. This interaction leads to PD-L1 deglycosylation, followed by its nuclear translocation via CMTM6-mediated transport [118].

Once translocated to the nucleus, PD-L1 can directly or indirectly interact with transcription factors and chromatin-modifying complexes to regulate gene expression (**Figure 6**). PD-L1 possesses specific binding motifs and canonical DNA-binding motifs of various transcription factors, including RelA (p65), STAT3, c-Jun, and IFN-regulatory factor (IRF) proteins [100, 114]. Among other functions, these transcription factors regulate gene expressions involved in immune-response pathways to modulate pro-inflammatory and anti-tumor immune response, specifically PD-L1 itself, PD-L2, V-domain immunoglobulin suppressor of T cell activation (VISTA), and

B7 homolog 3 (B7-H3) genes. In this context, nPD-L1 amplifies the activation of various immune-response pathways, establishing a positive feedback loop that further elevates PD-L1 expression and promotes immune evasion [100].

nPD-L1 also interacts with the transcription factor Sp1 (Specificity protein 1) and regulates the synthesis of Gas6 mRNA to promote Gas6 secretion. Gas6 is a ligand for receptor tyrosine kinases and activates the MerTK signaling pathway in the cell itself and in neighboring cells [99]. Activation of MerTK by its ligands triggers downstream signaling pathways—specially ERK, Akt and NF- κ B signaling—that promote cell proliferation, metastasis, and immune suppression within the TME [121–124]. Elevated MerTK expression is associated with higher poor prognosis, worse overall and disease-free survival, and disease progression in patients with GBM, [125], gastric cancer [126], colorectal cancer, [127] or melanoma [128].

The human Gasdermin (GSDM) protein family, comprising GSDMA, GSDMB, GSDMC, GSDMD, and GSDME, is the pivot of pyroptosis and shares a similar activation mechanism through cleavage by inflammation-activated caspases [129, 130]. Nuclear translocation of PD-L1 enhances the transcription of GSDMC and induces the switch from apoptosis to pyroptosis in cancer cells causing inflammation and tumor necrosis [114]. Another study shows that the transcription factor Sp1 directly interacts with the GSDME promoter and promotes GSDME transcription, leading to pyroptosis after chemotherapy [129]. However, whether this process depends on nPD-L1 translocation remains an open question.

Besides gene expression regulation, nPD-L1 is also involved in the regulation of other tumor-intrinsic processes like DNA double-strand-break (DSB) repair [111, 118]. Intracellular PD-L1 regulates the DNA damage response through multiple mechanisms, including binding to and stabilizing the mRNAs of DNA repair genes as well as serving as a key driver of DNA DSB repair via both the Non-Homologous End Joining (NHEJ) and Homologous Recombination (HR) pathways [111, 118]. NHEJ is initiated by the binding of a Ku protein heterodimer to DSBs, which serves as a platform for recruiting downstream repair factors [131]. nPD-L1 directly interacts with the core domains of Ku proteins, enhancing their binding to double-stranded DNA. This interaction also promotes the recruitment of additional NHEJ components, such as DNA-PKcs and XRCC4, thereby accelerating NHEJ-mediated repair and contributing to radio-resistance in human lung cancer cells [118]. In contrast, another study demonstrated that PD-L1 also facilitates HR by interacting with BARD1, thereby regulating BRCA1 nuclear localization [111]. Although the nuclear translocation of PD-L1 was not directly assessed, genetic depletion of PD-L1 reduced BRCA1 nuclear foci and impaired HR, as evidenced by decreased phosphorylation of RPA32 and ATR/Chk1—key markers of HR activity. Furthermore, PD-L1 depletion sensitized BRCA1-wildtype tumors to PARP inhibitors both in vitro and in vivo [111], which selectively kill tumors defective in homologous recombination DNA repair [132].

4.2 Cytoplasmic PD-L1 (cPD-L1)

cPD-L1 is a multifunctional hub that integrates pro-survival signaling, metabolic regulation, and autophagy, contributing to tumor progression and therapy resistance, independently of its role in immune evasion [96].

A key function of cPD-L1 is its capacity to shield tumor cells from the cytotoxic effects induced by chemotherapy and radiotherapy. cPD-L1 can bind to mRNAs like NBS1 and BRCA1, preventing their degradation and reducing cell death caused by DNA damage [133]. Additionally, cPD-L1 maintains activation of the cGAS-STING

pathway and low levels of IFN- β , which induce the expression of the interferon-related gene signature (IRGS)—a set of genes linked to cell survival and therapy resistance—leading to drug resistance [134].

Besides the role in evading cytotoxic effects of chemo and radiotherapy, it has been demonstrated cPD-L1 also regulates inflammatory pathways like COX-2 and STAT3, which support tumor growth. Studies show that anti-inflammatory drugs like resveratrol can reduce cPD-L1 levels in tumor cells, inhibiting their proliferation. cPD-L1 expression is controlled by STAT3 and COX-2 nuclear translocation, promoting tumor growth [135–137]. However, the exact molecular mechanisms behind cPD-L1's role in proliferation still need clarification.

Moreover, cPD-L1 has been implicated in the modulation of metabolic and autophagy-related pathways. It promotes basal activation of mTORC1 and alters mTORC2–Akt signaling in a context-dependent manner, which can impact the efficacy of mTOR inhibitors and autophagy modulators, positioning cPD-L1 as a potential biomarker for response to these therapies [105].

In gliomas, where hyperactivation of PI3K/Akt/mTOR signaling and resistance to standard therapies are hallmarks of disease, the role of cPD-L1 becomes particularly relevant. Its capacity to reinforce survival pathways may partially explain the limited efficacy of immune checkpoint blockade. Furthermore, emerging data suggest that glioma cells also utilize cPD-L1 to modulate their response to metabolic stress and DNA damage, contributing to treatment resistance in an immunologically “cold” microenvironment [138].

4.3 Extracellular space (sPD-L1)

Tumor cells and immune cells can release different forms of soluble PD-L1 (sPD-L1), including proteolytically cleaved fragments, secreted monomeric or dimeric splice variants, and PD-L1 associated with EVs such as microvesicles and exosomes (evPD-L1 and exoPD-L1). These soluble isoforms are detectable in serum or plasma samples and are gaining recognition as potential non-invasive biomarkers since levels of sPD-L1 may carry prognostic value. Elevated concentrations of circulating sPD-L1 prior to the initiation of immune checkpoint blockade have been correlated with reduced overall survival (OS) and progression-free survival (PFS) in solid cancers [139], suggesting an association with poorer clinical prognosis in cancer patients. In the same way, elevated plasma sPD-L1 levels in myelodysplastic syndrome patients are associated with disease progression and poorer OS [140]. On the other hand, changes in sPD-L1 levels during PD-1 inhibitor treatment do not significantly influence the prognosis of advanced NSCLC patients [141], suggesting that a continuous monitoring of sPD-L1 may not offer significant advantages compared to pre-treatment measurements.

exoPD-L1 shares the same membrane topology as mPD-L1, displaying its extracellular domain on the exosome's surface. In the same way, exoPD-L1 interacts with PD-1, and this binding can be blocked by antibodies that target PD-L1 [142].

It was demonstrated that GBM-derived EVs suppress T cell activation via PD-L1 in a subtype- and context-dependent manner. While PD-L1 high EVs directly inhibit T cells through PD1 binding, PD-L1 low EVs require IFN- γ to upregulate PD-L1 and exert similar effects. This inhibition is only partially reversed by PD1 blockade, implying the involvement of additional immunosuppressive mechanisms. exoPD-L1 binds PD1 in vitro and localizes to lymphocytes in vivo, underscoring its functional role. PD-L1 expression is enriched in mesenchymal GBM and IFN- γ -responsive

niches, and circulating exoPD-L1 correlates with tumor volume, supporting its potential as a non-invasive biomarker [143].

Interestingly, Zheng et al. showed that continuous low-dose TMZ stimulation promotes the generation of tumor stem cells, which interact with GBM cells to enhance TMZ resistance through the secretion of exoPD-L1, leading to activation of the AMPK/ULK1 pathway and induction of protective autophagy [144].

5. Conclusion

While PD-L1 has long been considered as a key immune checkpoint ligand operating at the tumor-immune interface, growing evidence reveals that its role extends far beyond PD-1 binding at the cell surface. The discovery of intracellular and extracellular PD-L1 variants, including cPD-L1, nPD-L1, exoPD-L1, and others, has shown a complex signaling landscape within cancer cells. These non-canonical forms contribute to tumor progression by modulating cell survival, metabolism, immune evasion, DNA repair, invasion, and even stemness, independently of T cell interaction.

Such multifunctionality challenges the current paradigm of immune checkpoint blockade and may help explain resistance mechanisms to anti-PD-1/PD-L1 therapies. By acting through the PI3K-Akt-mTOR pathway, interacting with intracellular kinases, or influencing transcriptional programs, PD-L1 emerges not only as a checkpoint but also as a signaling hub within tumor cells.

A deeper understanding of these intracellular and vesicle-associated PD-L1 opens new avenues for therapeutic intervention. Targeting PD-L1's intracellular signalosome or its trafficking apparatus could enhance current immunotherapies or offer alternatives in tumors refractory to surface PD-L1 blockade. Thus, PD-L1 could be a critical effector, actively orchestrating tumor cell survival from within.

Acknowledgements

This study was financed in part by the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul – FAPERGS (Edital 14/2022 ARD/RC #23/2551-0000812-2). Figure was created with the help of Biorender.com.

Conflict of interest

The authors declare no conflict of interest.

Author details

Thamiris Becker Scheffel^{1*} and Karine Rech Begnini²

1 Brain Institute, Pontifical Catholic University of Rio Grande do Sul,
Porto Alegre, RS, Brazil

2 Department of Biophysics, Federal University of Rio Grande do Sul,
Porto Alegre, RS, Brazil

*Address all correspondence to: t.scheffel@edu.pucrs.br

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO classification of tumors of the central nervous system: A summary. *Neuro-Oncology*. 2021;**23**:1231-1251. DOI: 10.1093/neuonc/noab106
- [2] Ostrom QT, Price M, Neff C, Cioffi G, Waite KA, Kruchko C, et al. CBTRUS statistical report: Primary brain and other central nervous system tumors diagnosed in the United States in 2015-2019. *Neuro-Oncology*. 2022;**24**:v1-v95. DOI: 10.1093/neuonc/noac202
- [3] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nature Reviews. Cancer*. 2012;**12**:252-264. DOI: 10.1038/nrc3239
- [4] Scheffell TB, Grave N, Vargas P, Diz FM, Rockenbach L, Morrone FB. Immunosuppression in gliomas via PD-1/PD-L1 axis and adenosine pathway. *Frontiers in Oncology*. 2020;**10**:617385. DOI: 10.3389/fonc.2020.617385
- [5] Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: The CheckMate 143 phase 3 randomized clinical trial. *JAMA Oncology*. 2020;**6**:1003-1010. DOI: 10.1001/jamaoncol.2020.1024
- [6] Cloughesy TF, Mochizuki AY, Orpilla JR, Hugo W, Lee AH, Davidson TB, et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. *Nature Medicine*. 2019;**25**:477-486. DOI: 10.1038/s41591-018-0337-7
- [7] Berghoff AS, Kiesel B, Widhalm G, Rajky O, Ricken G, Wöhrer A, et al. Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. *Neuro-Oncology*. 2015;**17**:1064-1075. DOI: 10.1093/neuonc/nou307
- [8] Xiong W, Gao Y, Wei W, Zhang J. Extracellular and nuclear PD-L1 in modulating cancer immunotherapy. *Trends in Cancer*. 2021;**7**:837-846. DOI: 10.1016/j.trecan.2021.03.003
- [9] Azuma T, Yao S, Zhu G, Flies AS, Flies SJ, Chen L. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. *Blood*. 2008;**111**:3635-3643. DOI: 10.1182/blood-2007-11-123141
- [10] Li G, Qin Z, Chen Z, Xie L, Wang R, Zhao H. Tumor microenvironment in treatment of glioma. *OpenMed*. 2017;**12**:247-251. DOI: 10.1515/med-2017-0035
- [11] Hambardzumyan D, Bergers G. Glioblastoma: Defining tumor niches. *Trends in Cancer*. 2015;**1**:252-265. DOI: 10.1016/j.trecan.2015.10.009
- [12] Barthel L, Hadamitzky M, Dammann P, Schedlowski M, Sure U, Thakur BK, et al. Glioma: Molecular signature and crossroads with tumor microenvironment. *Cancer Metastasis Reviews*. 2021;**41**(1):53-75. DOI: 10.1007/s10555-021-09997-9
- [13] Irvin DM, McNeill RS, Bash RE, Miller CR. Intrinsic astrocyte heterogeneity influences tumor growth in glioma mouse models. *Brain Pathology (Zurich, Switzerland)*. 2017;**27**:36-50. DOI: 10.1111/bpa.12348
- [14] Brandao M, Simon T, Critchley G, Giamas G. Astrocytes, the rising stars of the glioblastoma microenvironment.

Glia. 2019;**67**:779-790. DOI: 10.1002/glia.23520

[15] O'Brien ER, Howarth C, Sibson NR. The role of astrocytes in CNS tumors: Pre-clinical models and novel imaging approaches. *Frontiers in Cellular Neuroscience*. 2013;**7**:40. DOI: 10.3389/fncel.2013.00040

[16] Chen W, Wang D, Du X, He Y, Chen S, Shao Q, et al. Glioma cells escaped from cytotoxicity of temozolomide and vincristine by communicating with human astrocytes. *Medical Oncology (Northwood, London, England)*. 2015;**32**:43. DOI: 10.1007/s12032-015-0487-0

[17] Chen Z, Hambardzumyan D. Immune microenvironment in glioblastoma subtypes. *Frontiers in Immunology*. 2018;**9**:1004. DOI: 10.3389/fimmu.2018.01004

[18] Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nature Reviews. Clinical Oncology*. 2017;**14**:717-734. DOI: 10.1038/nrclinonc.2017.101

[19] Wu T, Dai Y. Tumor microenvironment and therapeutic response. *Cancer Letters*. 2017;**387**:61-68. DOI: 10.1016/j.canlet.2016.01.043

[20] Charles NA, Holland EC, Gilbertson R, Glass R, Kettenmann H. The brain tumor microenvironment. *Glia*. 2011;**59**:1169-1180. DOI: 10.1002/glia.21136

[21] Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression. *Nature Neuroscience*. 2016;**19**:20-27. DOI: 10.1038/nn.4185

[22] Guadagno E, Presta I, Maisano D, Donato A, Pirrone CK, Cardillo G, et al.

Role of macrophages in brain tumor growth and progression. *International Journal of Molecular Sciences*. 2018;**19**(4):1005. DOI: 10.3390/ijms19041005

[23] Lambrechts D, Wauters E, Boeckx B, Aibar S, Nittner D, Burton O, et al. Phenotype molding of stromal cells in the lung tumor microenvironment. *Nature Medicine*. 2018;**24**:1277-1289. DOI: 10.1038/s41591-018-0096-5

[24] Azizi E, Carr AJ, Plitas G, Cornish AE, Konopacki C, Prabhakaran S, et al. Single-cell map of diverse immune phenotypes in the breast tumor microenvironment. *Cell*. 2018;**174**:1293-1308.e36. DOI: 10.1016/j.cell.2018.05.060

[25] Sathe A, Grimes SM, Lau BT, Chen J, Suarez C, Huang RJ, et al. Single-cell genomic characterization reveals the cellular reprogramming of the gastric tumor microenvironment. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2020;**26**:2640-2653. DOI: 10.1158/1078-0432.CCR-19-3231

[26] Müller S, Kohanbash G, Liu SJ, Alvarado B, Carrera D, Bhaduri A, et al. Single-cell profiling of human gliomas reveals macrophage ontogeny as a basis for regional differences in macrophage activation in the tumor microenvironment. *Genome Biology*. 2017;**18**:234. DOI: 10.1186/s13059-017-1362-4

[27] Konduri SD, Rao CN, Chandrasekar N, Tasiou A, Mohanam S, Kin Y, et al. A novel function of tissue factor pathway inhibitor-2 (TFPI-2) in human glioma invasion. *Oncogene*. 2001;**20**:6938-6945. DOI: 10.1038/sj.onc.1204847

[28] Takano T, Lin JH, Arcuino G, Gao Q, Yang J, Nedergaard M. Glutamate release

- promotes growth of malignant gliomas. *Nature Medicine*. 2001;7:1010-1015. DOI: 10.1038/nm0901-1010
- [29] Cuddapah VA, Robel S, Watkins S, Sontheimer H. A neurocentric perspective on glioma invasion. *Nature Reviews. Neuroscience*. 2014;15:455-465. DOI: 10.1038/nrn3765
- [30] Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, et al. Cell migration: Integrating signals from front to back. *Science*. 2003;302:1704-1709. DOI: 10.1126/science.1092053
- [31] Kwiatkowska A, Symons M. Signaling determinants of glioma cell invasion. In: Barañska J, editor. *Glioma Signal*. Dordrecht: Springer Netherlands; 2013. pp. 121-141. DOI: 10.1007/978-94-007-4719-7_7
- [32] Wippold FJ, Lämmle M, Anatelli F, Lennerz J, Perry A. Neuropathology for the neuroradiologist: Palisades and pseudopalisades. *AJNR*. *American Journal of Neuroradiology*. 2006;27:2037-2041
- [33] Korkolopoulou P, Patsouris E, Konstantinidou AE, Pavlopoulos PM, Kavantzias N, Boviatsis E, et al. Hypoxia-inducible factor 1 α /vascular endothelial growth factor axis in astrocytomas. Associations with microvessel morphometry, proliferation and prognosis. *Neuropathology and Applied Neurobiology*. 2004;30:267-278. DOI: 10.1111/j.1365-2990.2003.00535.x
- [34] Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P, et al. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1 α . *Science*. 2009;324:261-265. DOI: 10.1126/science.1170944
- [35] Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell*. 2012;148:399-408. DOI: 10.1016/j.cell.2012.01.021
- [36] Brat DJ, Castellano-Sanchez AA, Hunter SB, Pecot M, Cohen C, Hammond EH, et al. Pseudopalisades in glioblastoma are hypoxic, express extracellular matrix proteases, and are formed by an actively migrating cell population. *Cancer Research*. 2004;64:920-927. DOI: 10.1158/0008-5472.can-03-2073
- [37] Zagzag D, Lukyanov Y, Lan L, Ali MA, Esencay M, Mendez O, et al. Hypoxia-inducible factor 1 and VEGF upregulate CXCR4 in glioblastoma: Implications for angiogenesis and glioma cell invasion. *Laboratory Investigation; A Journal of Technical Methods and Pathology*. 2006;86:1221-1232. DOI: 10.1038/labinvest.3700482
- [38] Brat DJ. Glioblastoma: Biology, genetics, and behavior. *American Society of Clinical Oncology Educational Book*. 2012:102-107. DOI: 10.14694/EdBook_AM.2012.32.48
- [39] Balkwill F, Mantovani A. Inflammation and cancer: Back to Virchow? *Lancet (London, England)*. 2001;357:539-545. DOI: 10.1016/S0140-6736(00)04046-0
- [40] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell*. 2011;144:646-674. DOI: 10.1016/j.cell.2011.02.013
- [41] Zugazagoitia J, Guedes C, Ponce S, Ferrer I, Molina-Pinelo S, Paz-Ares L. Current challenges in cancer treatment. *Clinical Therapeutics*. 2016;38:1551-1566. DOI: 10.1016/j.clinthera.2016.03.026
- [42] D'Alessio A, Proietti G, Sica G, Scicchitano BM. Pathological and molecular features of glioblastoma and its peritumoral tissue. *Cancers*.

2019;**11**:469. DOI: 10.3390/cancers11040469

[43] Hao C, Parney IF, Roa WH, Turner J, Petruk KC, Ramsay DA. Cytokine and cytokine receptor mRNA expression in human glioblastomas: Evidence of Th1, Th2 and Th3 cytokine dysregulation. *Acta Neuropathologica* (Berlin). 2002;**103**:171-178. DOI: 10.1007/s004010100448

[44] Gieryng A, Pszczolkowska D, Walentynowicz KA, Rajan WD, Kaminska B. Immune microenvironment of gliomas. *Laboratory Investigation; A Journal of Technical Methods and Pathology*. 2017;**97**:498-518. DOI: 10.1038/labinvest.201719

[45] Tankov S, Walker PR. Glioma-derived extracellular vesicles - Far more than local mediators. *Frontiers in Immunology*. 2021;**12**:679954. DOI: 10.3389/fimmu.2021.679954

[46] Mantovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial cell function. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*. 1992;**6**:2591-2599. DOI: 10.1096/fasebj.6.8.1592209

[47] Condeelis J, Pollard JW. Macrophages: Obligate partners for tumor cell migration, invasion, and metastasis. *Cell*. 2006;**124**:263-266. DOI: 10.1016/j.cell.2006.01.007

[48] Sica A, Bronte V. Altered macrophage differentiation and immune dysfunction in tumor development. *The Journal of Clinical Investigation*. 2007;**117**:1155-1166. DOI: 10.1172/JCI31422

[49] Kaminska B, Kocyk M, Kijewska M. TGF beta signaling and its role in glioma pathogenesis. *Advances in Experimental*

Medicine and Biology. 2013;**986**:171-187. DOI: 10.1007/978-94-007-4719-7_9

[50] Woroniecka KI, Rhodin KE, Chongsathidkiet P, Keith KA, Fecci PE. T-cell dysfunction in glioblastoma: Applying a new framework. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2018;**24**:3792-3802. DOI: 10.1158/1078-0432.CCR-18-0047

[51] Takimoto T, Wakabayashi Y, Sekiya T, Inoue N, Morita R, Ichiyama K, et al. Smad2 and Smad3 are redundantly essential for the TGF-beta-mediated regulation of regulatory T plasticity and Th1 development. *Journal of Immunology* (Baltimore, Md.: 1950). 2010;**185**:842-855. DOI: 10.4049/jimmunol.0904100

[52] Walker MR, Kasprovicz DJ, Gersuk VH, Bènard A, Van Landeghen M, Buckner JH, et al. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25- T cells. *The Journal of Clinical Investigation*. 2003;**112**:1437-1443. DOI: 10.1172/JCI200319441

[53] Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *The Journal of Experimental Medicine*. 2009;**206**:3015-3029. DOI: 10.1084/jem.20090847

[54] Wainwright DA, Sengupta S, Han Y, Lesniak MS. Thymus-derived rather than tumor-induced regulatory T cells predominate in brain tumors. *Neuro-Oncology*. 2011;**13**:1308-1323. DOI: 10.1093/neuonc/nor134

[55] Wainwright DA, Dey M, Chang A, Lesniak MS. Targeting Tregs in malignant brain cancer:

- Overcoming IDO. *Frontiers in Immunology*. 2013;**4**:116. DOI: 10.3389/fimmu.2013.00116
- [56] Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. *Nature Reviews. Immunology*. 2010;**10**:490-500. DOI: 10.1038/nri2785
- [57] Takeuchi Y, Nishikawa H. Roles of regulatory T cells in cancer immunity. *International Immunology*. 2016;**28**:401-409. DOI: 10.1093/intimm/dxw025
- [58] deLeeuw RJ, Kost SE, Kakal JA, Nelson BH. The prognostic value of FoxP3+ tumor-infiltrating lymphocytes in cancer: A critical review of the literature. *Clinical Cancer Research*. 2012;**18**:3022-3029. DOI: 10.1158/1078-0432.CCR-11-3216
- [59] Lathia JD, Mack SC, Mulkearns-Hubert EE, Valentim CLL, Rich JN. Cancer stem cells in glioblastoma. *Genes & Development*. 2015;**29**:1203-1217. DOI: 10.1101/gad.261982.115
- [60] Riley JL. PD-1 signaling in primary T cells. *Immunological Reviews*. 2009;**229**:114-125. DOI: 10.1111/j.1600-065X.2009.00767.x
- [61] Jiang X, Wang J, Deng X, Xiong F, Ge J, Xiang B, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. *Molecular Cancer*. 2019;**18**:10. DOI: 10.1186/s12943-018-0928-4
- [62] Escors D, Gato-Cañas M, Zuazo M, Arasanz H, García-Granda MJ, Vera R, et al. The intracellular signalosome of PD-L1 in cancer cells. *Signal Transduction and Targeted Therapy*. 2018;**3**:26. DOI: 10.1038/s41392-018-0022-9
- [63] Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunological Reviews*. 2010;**236**:219-242. DOI: 10.1111/j.1600-065X.2010.00923.x
- [64] Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annual Review of Immunology*. 2008;**26**:677-704. DOI: 10.1146/annurev.immunol.26.021607.090331
- [65] Bloch O, Crane CA, Kaur R, Safaee M, Rutkowski MJ, Parsa AT. Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2013;**19**:3165-3175. DOI: 10.1158/1078-0432.CCR-12-3314
- [66] Zeng J, See AP, Phallen J, Jackson CM, Belcaid Z, Ruzevick J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. *International Journal of Radiation Oncology, Biology, Physics*. 2013;**86**:343-349. DOI: 10.1016/j.ijrobp.2012.12.025
- [67] Xue S, Hu M, Iyer V, Yu J. Blocking the PD-1/PD-L1 pathway in glioma: A potential new treatment strategy. *Journal of Hematology & Oncology*. 2017;**10**(1):81. DOI: 10.1186/s13045-017-0455-6
- [68] Kurz SC, Wen PY. Quo Vadis—Do immunotherapies have a role in glioblastoma? *Current Treatment Options in Neurology*. 2018;**20**:14. DOI: 10.1007/s11940-018-0499-0
- [69] Garcia-Diaz A, Shin DS, Moreno BH, Saco J, Escuin-Ordinas H, Rodriguez GA, et al. Interferon receptor signaling pathways regulating PD-L1 and PD-L2 expression. *Cell Reports*. 2017;**19**:1189-1201. DOI: 10.1016/j.celrep.2017.04.031
- [70] Gowrishankar K, Gunatilake D, Gallagher SJ, Tiffen J, Rizos H, Hersey P.

Inducible but not constitutive expression of PD-L1 in human melanoma cells is dependent on activation of NF- κ B. *PLoS One*. 2015;**10**:e0123410. DOI: 10.1371/journal.pone.0123410

[71] Wintterle S, Schreiner B, Mitsdoerffer M, Schneider D, Chen L, Meyermann R, et al. Expression of the B7-related molecule B7-H1 by glioma cells: A potential mechanism of immune paralysis. *Cancer Research*. 2003;**63**:7462-7467

[72] Yao Y, Tao R, Wang X, Wang Y, Mao Y, Zhou LF. B7-H1 is correlated with malignancy-grade gliomas but is not expressed exclusively on tumor stem-like cells. *Neuro-Oncology*. 2009;**11**:757-766. DOI: 10.1215/15228517-2009-014

[73] Nduom EK, Wei J, Yaghi NK, Huang N, Kong L-Y, Gabrusiewicz K, et al. PD-L1 expression and prognostic impact in glioblastoma. *Neuro-Oncology*. 2016;**18**:195-205. DOI: 10.1093/neuonc/nov172

[74] Litak J, Mazurek M, Grochowski C, Kamieniak P, Roliński J. PD-L1/PD-1 axis in glioblastoma multiforme. *International Journal of Molecular Sciences*. 2019;**20**:E5347. DOI: 10.3390/ijms20215347

[75] Wilmotte R, Burkhardt K, Kindler V, Belkouch M-C, Dussex G, de Tribolet N, et al. B7-homolog 1 expression by human glioma: A new mechanism of immune evasion. *Neuroreport*. 2005;**16**:1081-1085. DOI: 10.1097/00001756-200507130-00010

[76] Yadollahi P, Jeon Y-K, Ng WL, Choi I. Current understanding of cancer-intrinsic PD-L1: Regulation of expression and its protumoral activity. *BMB Reports*. 2021;**54**:12-20. DOI: 10.5483/BMBRep.2021.54.1.241

[77] Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nature Medicine*. 2007;**13**:84-88. DOI: 10.1038/nm1517

[78] Noman MZ, Desantis G, Janji B, Hasmim M, Karay S, Dessen P, et al. PD-L1 is a novel direct target of HIF-1 α , and its blockade under hypoxia enhanced MDSC-mediated T cell activation. *The Journal of Experimental Medicine*. 2014;**211**:781-790. DOI: 10.1084/jem.20131916

[79] Gong A-Y, Zhou R, Hu G, Li X, Splinter PL, O'Hara SP, et al. MicroRNA-513 regulates B7-H1 translation and is involved in IFN-gamma-induced B7-H1 expression in cholangiocytes. *Journal of Immunology* (Baltimore, Md.: 1950). 2009;**182**:1325-1333. DOI: 10.4049/jimmunol.182.3.1325

[80] Wang Y, Wang D, Xie G, Yin Y, Zhao E, Tao K, et al. MicroRNA-152 regulates immune response via targeting B7-H1 in gastric carcinoma. *Oncotarget*. 2017;**8**:28125-28134. DOI: 10.18632/oncotarget.15924

[81] Kataoka K, Shiraishi Y, Takeda Y, Sakata S, Matsumoto M, Nagano S, et al. Aberrant PD-L1 expression through 3'-UTR disruption in multiple cancers. *Nature*. 2016;**534**:402-406. DOI: 10.1038/nature18294

[82] Burr ML, Sparbier CE, Chan Y-C, Williamson JC, Woods K, Beavis PA, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature*. 2017;**549**:101-105. DOI: 10.1038/nature23643

[83] Mezzadra R, Sun C, Jae LT, Gomez-Eerland R, de Vries E, Wu W, et al. Identification of CMTM6 and CMTM4

as PD-L1 protein regulators. *Nature*. 2017;**549**:106-110. DOI: 10.1038/nature23669

[84] Bedke T, Pretsch L, Karakhanova S, Enk AH, Mahnke K. Endothelial cells augment the suppressive function of CD4⁺CD25⁺Foxp3⁺ regulatory T cells: Involvement of programmed death-1 and IL-10. *Journal of Immunology*. 2010;**184**:5562-5570. DOI: 10.4049/jimmunol.0902458

[85] Pedoeem A, Azoulay-Alfaguter I, Strazza M, Silverman GJ, Mor A. Programmed death-1 pathway in cancer and autoimmunity. *Clinical Immunology (Orlando, Fla.)*. 2014;**153**:145-152. DOI: 10.1016/j.clim.2014.04.010

[86] Janse van Rensburg HJ, Azad T, Ling M, Hao Y, Snetsinger B, Khanal P, et al. The hippo pathway component TAZ promotes immune evasion in human cancer through PD-L1. *Cancer Research*. 2018;**78**:1457-1470. DOI: 10.1158/0008-5472.CAN-17-3139

[87] Wei F, Zhong S, Ma Z, Kong H, Medvec A, Ahmed R, et al. Strength of PD-1 signaling differentially affects T-cell effector functions. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;**110**:E2480-E2489. DOI: 10.1073/pnas.1305394110

[88] Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy for cancer: An overview of FDA-approved immune checkpoint inhibitors. *International Immunopharmacology*. 2018;**62**:29-39. DOI: 10.1016/j.intimp.2018.06.001

[89] Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. 2012;**366**(26):2443-2454. DOI: 10.1056/NEJMoa1200690

[90] Weber J, D'Angelo S, Minor D, Hodi F, Gutzmer R, Neyns B, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): A randomised, controlled, open-label, phase 3 trial. *The Lancet Oncology*. 2015;**16**(4):375-384. DOI: 10.1016/S1470-2045(15)70076-8

[91] Lee EQ. Immune checkpoint inhibitors in GBM. *Journal of Neuro-Oncology*. 2021;**155**:1-11. DOI: 10.1007/s11060-021-03859-8

[92] Lou Y, Shi J, Guo D, Qureshi AK, Song L. Function of PD-L1 in antitumor immunity of glioma cells. *Saudi Journal of Biological Sciences*. 2017;**24**:803-807. DOI: 10.1016/j.sjbs.2015.06.025

[93] O'Donnell JS, Long GV, Scolyer RA, Teng MWL, Smyth MJ. Resistance to PD1/PDL1 checkpoint inhibition. *Cancer Treatment Reviews*. 2017;**52**:71-81. DOI: 10.1016/j.ctrv.2016.11.007

[94] Chang C-H, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell*. 2015;**162**:1229-1241. DOI: 10.1016/j.cell.2015.08.016

[95] Sun C, Mezzadra R, Schumacher TN. Regulation and function of the PD-L1 checkpoint. *Immunity*. 2018;**48**:434-452. DOI: 10.1016/j.immuni.2018.03.014

[96] Wang Y, Zhou Y, Yang L, Lei L, He B, Cao J, et al. Challenges coexist with opportunities: Spatial heterogeneity expression of PD-L1 in cancer therapy. *Advancement of Science*. 2023;**11**:2303175. DOI: 10.1002/adv.202303175

[97] Hanks BA. The "inside" story on tumor-expressed PD-L1. *Cancer Research*. 2022;**82**:2069-2071. DOI: 10.1158/0008-5472.CAN-22-1060

- [98] Ying H, Zhang X, Duan Y, Lao M, Xu J, Yang H, et al. Non-cytomembrane PD-L1: An atypical target for cancer. *Pharmacological Research*. 2021;**170**:105741. DOI: 10.1016/j.phrs.2021.105741
- [99] Du W, Zhu J, Zeng Y, Liu T, Zhang Y, Cai T, et al. KPNB1-mediated nuclear translocation of PD-L1 promotes non-small cell lung cancer cell proliferation via the Gas6/MerTK signaling pathway. *Cell Death and Differentiation*. 2021;**28**:1284-1300. DOI: 10.1038/s41418-020-00651-5
- [100] Gao Y, Nihira NT, Bu X, Chu C, Zhang J, Kolodziejczyk A, et al. Acetylation-dependent regulation of PD-L1 nuclear translocation dictates the efficacy of anti-PD-1 immunotherapy. *Nature Cell Biology*. 2020;**22**:1064-1075. DOI: 10.1038/s41556-020-0562-4
- [101] Zhu K, Huang W, Wang W, Liao L, Li S, Yang S, et al. Up-regulation of S100A4 expression by HBx protein promotes proliferation of hepatocellular carcinoma cells and its correlation with clinical survival. *Gene*. 2020;**749**:144679. DOI: 10.1016/j.gene.2020.144679
- [102] Qu Q-X, Xie F, Huang Q, Zhang X-G. Membranous and cytoplasmic expression of PD-L1 in ovarian cancer cells. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*. 2017;**43**:1893-1906. DOI: 10.1159/000484109
- [103] Schulz D, Streller M, Piendl G, Brockhoff G, Reichert TE, Menevse AN, et al. Differential localization of PD-L1 and Akt-1 involvement in radioresistant and radiosensitive cell lines of head and neck squamous cell carcinoma. *Carcinogenesis*. 2020;**41**:984-992. DOI: 10.1093/carcin/bgz177
- [104] Palmer CS, Ostrowski M, Balderson B, Christian N, Crowe SM. Glucose metabolism regulates T cell activation, differentiation, and functions. *Frontiers in Immunology*. 2015;**6**:1. DOI: 10.3389/fimmu.2015.00001
- [105] Clark CA, Gupta HB, Sareddy G, Pandeswara S, Lao S, Yuan B, et al. Tumor-intrinsic PD-L1 signals regulate cell growth, pathogenesis, and autophagy in ovarian cancer and melanoma. *Cancer Research*. 2016;**76**:6964-6974. DOI: 10.1158/0008-5472.CAN-16-0258
- [106] Chen RQ, Xu XH, Liu F, Li CY, Li YJ, Li XR, et al. The binding of PD-L1 and Akt facilitates glioma cell invasion upon starvation via Akt/autophagy/F-actin signaling. *Frontiers in Oncology*. 2019;**9**:1347. DOI: 10.3389/fonc.2019.01347
- [107] Chen J, Zhang XD, Proud C. Dissecting the signaling pathways that mediate cancer in PTEN and LKB1 double-knockout mice. *Science Signaling*. 2015;**8**:pe1-pe1. DOI: 10.1126/scisignal.aac8321
- [108] Almozyan S, Colak D, Mansour F, Alaiya A, Al-Harazi O, Qattan A, et al. PD-L1 promotes OCT4 and Nanog expression in breast cancer stem cells by sustaining PI3K/AKT pathway activation. *International Journal of Cancer*. 2017;**141**:1402-1412. DOI: 10.1002/ijc.30834
- [109] Liu Y, Carlsson R, Ambjørn M, Hasan M, Badn W, Darabi A, et al. PD-L1 expression by neurons nearby tumors indicates better prognosis in glioblastoma patients. *The Journal of Neuroscience*. 2013;**33**:14231-14245. DOI: 10.1523/JNEUROSCI.5812-12.2013
- [110] Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al.

Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nature Medicine*. 2002;**8**:793-800. DOI: 10.1038/nm730

[111] Kornepati AVR, Boyd JT, Murray CE, Saifetiarova J, de la Peña AB, Rogers CM, et al. Tumor intrinsic PD-L1 promotes DNA repair in distinct cancers and suppresses PARP inhibitor-induced synthetic lethality. *Cancer Research*. 2022;**82**:2156-2170. DOI: 10.1158/0008-5472.CAN-21-2076

[112] Lee D, Cho M, Kim E, Seo Y, Cha J-H. PD-L1: From cancer immunotherapy to therapeutic implications in multiple disorders. *Molecular Therapy*. 2024;**32**:4235-4255. DOI: 10.1016/j.ymthe.2024.09.026

[113] Polioudaki H, Chantziou A, Kalyvianaki K, Malamos P, Notas G, Mavroudis D, et al. Nuclear localization of PD-L1: Artifact or reality? *Cellular Oncology (Dordrecht, Netherlands)*. 2019;**42**:237-242. DOI: 10.1007/s13402-018-00419-7

[114] Hou J, Zhao R, Xia W, Chang C-W, You Y, Hsu J-M, et al. PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumour necrosis. *Nature Cell Biology*. 2020;**22**:1264-1275. DOI: 10.1038/s41556-020-0575-z

[115] Yu J, Qin B, Moyer AM, Nowsheen S, Tu X, Dong H, et al. Regulation of sister chromatid cohesion by nuclear PD-L1. *Cell Research*. 2020;**30**:590-601. DOI: 10.1038/s41422-020-0315-8

[116] Yu J, Zhuang A, Gu X, Hua Y, Yang L, Ge S, et al. Nuclear PD-L1 promotes EGR1-mediated angiogenesis and accelerates tumorigenesis. *Cell Discovery*. 2023;**9**:1-19. DOI: 10.1038/s41421-023-00521-7

[117] Cha J-H, Yang W-H, Xia W, Wei Y, Chan L-C, Lim S-O, et al. Metformin promotes antitumor immunity via endoplasmic-reticulum-associated degradation of PD-L1. *Molecular Cell*. 2018;**71**:606-620.e7. DOI: 10.1016/j.molcel.2018.07.030

[118] Shu Z, Dwivedi B, Switchenko JM, Yu DS, Deng X. PD-L1 deglycosylation promotes its nuclear translocation and accelerates DNA double-strand-break repair in cancer. *Nature Communications*. 2024;**15**:6830. DOI: 10.1038/s41467-024-51242-8

[119] Horita H, Law A, Hong S, Middleton K. Identifying regulatory posttranslational modifications of PD-L1: A focus on monoubiquitination. *Neoplasia*. 2017;**19**:346-353. DOI: 10.1016/j.neo.2017.02.006

[120] Ghebeh H, Lehe C, Barhoush E, Al-Romaih K, Tulbah A, Al-Alwan M, et al. Doxorubicin downregulates cell surface B7-H1 expression and upregulates its nuclear expression in breast cancer cells: Role of B7-H1 as an anti-apoptotic molecule. *Breast Cancer Research: BCR*. 2010;**12**:R48. DOI: 10.1186/bcr2605

[121] Wu G, Ma Z, Cheng Y, Hu W, Deng C, Jiang S, et al. Targeting Gas6/TAM in cancer cells and tumor microenvironment. *Molecular Cancer*. 2018;**17**:20. DOI: 10.1186/s12943-018-0769-1

[122] van der Meer JHM, van der Poll T, van 't Veer C. TAM receptors, Gas6, and protein S: Roles in inflammation and hemostasis. *Blood*. 2014;**123**:2460-2469. DOI: 10.1182/blood-2013-09-528752

[123] Myers KV, Amend SR, Pienta KJ. Targeting Tyro3, Axl and MerTK (TAM receptors): Implications for macrophages in the tumor microenvironment.

Molecular Cancer. 2019;**18**:94.
DOI: 10.1186/s12943-019-1022-2

[124] Eom H, Kaushik N, Yoo K-C, Shim J-K, Kwon M, Choi M-Y, et al. MerTK mediates STAT3-KRAS/SRC-signaling axis for glioma stem cell maintenance. *Artificial Cells, Nanomedicine, and Biotechnology*. 2018;**46**:87-95. DOI: 10.1080/21691401.2018.1452022

[125] Wu J, Frady LN, Bash RE, Cohen SM, Schorzman AN, Su Y-T, et al. MerTK as a therapeutic target in glioblastoma. *Neuro-Oncology*. 2018;**20**:92-102. DOI: 10.1093/neuonc/nox111

[126] Yi JH, Jang J, Cho J, Do I-G, Hong M, Kim ST, et al. MerTK is a novel therapeutic target in gastric cancer. *Oncotarget*. 2017;**8**:96656-96667. DOI: 10.18632/oncotarget.3750

[127] Frejno M, Zenezini Chiozzi R, Wilhelm M, Koch H, Zheng R, Klaeager S, et al. Pharmacoproteomic characterisation of human colon and rectal cancer. *Molecular Systems Biology*. 2017;**13**:951. DOI: 10.15252/msb.20177701

[128] Schlegel J, Sambade MJ, Sather S, Moschos SJ, Tan A-C, Winges A, et al. MERTK receptor tyrosine kinase is a therapeutic target in melanoma. *The Journal of Clinical Investigation*. 2013;**123**:2257-2267. DOI: 10.1172/jci67816

[129] Pan J, Li Y, Gao W, Jiang Q, Geng L, Ding J, et al. Transcription factor Sp1 transcriptionally enhances GSDME expression for pyroptosis. *Cell Death & Disease*. 2024;**15**:1-8. DOI: 10.1038/s41419-024-06455-6

[130] Kovacs SB, Miao EA. Gasdermins: Effectors of pyroptosis. *Trends in Cell Biology*. 2017;**27**:673-684. DOI: 10.1016/j.tcb.2017.05.005

[131] Rinaldi C, Pizzul P, Casari E, Mangiagalli M, Tisi R, Longhese MP. The Ku complex promotes DNA end-bridging and this function is antagonized by Tel1/ATM kinase. *Nucleic Acids Research*. 2023;**51**:1783-1802. DOI: 10.1093/nar/gkad062

[132] Farmer H, McCabe N, Lord CJ, Tutt ANJ, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. 2005;**434**:917-921. DOI: 10.1038/nature03445

[133] Tu X, Qin B, Zhang Y, Zhang C, Kahila M, Nowsheen S, et al. PD-L1 (B7-H1) competes with the RNA exosome to regulate the DNA damage response and can be targeted to sensitize to radiation or chemotherapy. *Molecular Cell*. 2019;**74**:1215-1226.e4. DOI: 10.1016/j.molcel.2019.04.005

[134] Cheon H, Holvey-Bates EG, McGrail DJ, Stark GR. PD-L1 sustains chronic, cancer cell-intrinsic responses to type I interferon, enhancing resistance to DNA damage. *Proceedings of the National Academy of Sciences of the United States of America*. 2021;**118**:e2112258118. DOI: 10.1073/pnas.2112258118

[135] Chen Y-R, Chen Y-S, Chin Y-T, Li Z-L, Shih Y-J, Yang Y-CSH, et al. Thyroid hormone-induced expression of inflammatory cytokines interfere with resveratrol-induced anti-proliferation of oral cancer cells. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*. 2019;**132**:110693. DOI: 10.1016/j.fct.2019.110693

[136] Chin Y-T, Wei P-L, Ho Y, Nana AW, Changou CA, Chen Y-R, et al. Thyroxine inhibits resveratrol-caused apoptosis by PD-L1 in ovarian cancer cells.

Endocrine-Related Cancer. 2018;**25**:533-545. DOI: 10.1530/ERC-17-0376

[137] Ho Y, Chen Y-F, Wang L-H, Hsu K-Y, Chin Y-T, Yang Y-CSH, et al. Inhibitory effect of anoectochilus formosanus extract on hyperglycemia-related PD-L1 expression and cancer proliferation. *Frontiers in Pharmacology*. 2018;**9**:807. DOI: 10.3389/fphar.2018.00807

[138] Chen RQ, Xu XH, Liu F, Li CY, Li YJ, Li XR, et al. The binding of PD-L1 and Akt facilitates glioma cell invasion upon starvation via Akt/autophagy/Factin signaling. *Frontiers in Oncology*. 2019;**9**:1347. DOI: 10.3389/fonc.2019.01347

[139] Scirocchi F, Strigari L, Di Filippo A, Napoletano C, Pace A, Rahimi H, et al. Soluble PD-L1 as a prognostic factor for immunotherapy treatment in solid tumors: Systematic review and meta-analysis. *International Journal of Molecular Sciences*. 2022;**23**:14496. DOI: 10.3390/ijms232214496

[140] Yang X, Jiang L, Zhang X, Peng J, Qian H, Huang L, et al. Soluble PD-L1 as a novel biomarker predicts poor outcomes and disease progression in de novo myelodysplastic syndromes. *Biomarker Research*. 2024;**12**:115. DOI: 10.1186/s40364-024-00665-y

[141] Shimizu T, Inoue E, Ohkuma R, Kobayashi S, Tsunoda T, Wada S. Soluble PD-L1 changes in advanced non-small cell lung cancer patients treated with PD-1 inhibitors: An individual patient data meta-analysis. *Frontiers in Immunology*. 2023;**14**:1308381. DOI: 10.3389/fimmu.2023.1308381

[142] Chen G, Huang AC, Zhang W, Zhang G, Wu M, Xu W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature*. 2018;**560**:382-386. DOI: 10.1038/s41586-018-0392-8

[143] Ricklefs FL, Alayo Q, Krenzlin H, Mahmoud AB, Speranza MC, Nakashima H, et al. Immune evasion mediated by PD-L1 on glioblastoma-derived extracellular vesicles. *Science Advances*. 2018;**4**:eaar2766. DOI: 10.1126/sciadv.aar2766

[144] Zheng Y, Liu L, Wang Y, Xiao S, Mai R, Zhu Z, et al. Glioblastoma stem cell (GSC)-derived PD-L1-containing exosomes activates AMPK/ULK1 pathway mediated autophagy to increase temozolomide-resistance in glioblastoma. *Cell & Bioscience*. 2021;**11**:63. DOI: 10.1186/s13578-021-00575-8

The Tumor Microenvironment: Immunological Interactions and Therapeutic Targeting

Anca-Florina Zgura, Rodica-Maricela Anghel and Stefania Chipuc

Abstract

A fundamental concern among oncologists is related to the identification of how the tumor microenvironment (*TME*) influences the outcome of a patient. *TME* influences all processes linked with tumors: tumor growth, evolution, progression, metastasis, and, finally, treatment response. By targeting different elements of *TME*, we, as scientists, can control the tumoral landscape. There are four elements within *TME*: different types of cells, such as tumor endothelial cells and cancer-associated fibroblasts; soluble factors such as chemokines and cytokines; growth factors; and their reciprocal interactions. This allows us to develop novel therapeutic strategies that alter only the cancerous cell or the supporting ones, avoiding the healthy tissues. In this chapter, the focus will be on *TME*'s components and their role inside the tumoral space and on how we can manipulate them to reach our therapeutic goals.

Keywords: cancer, tumor microenvironment, cellular element, reciprocal interactions, targeted therapy, immunotherapy, immune checkpoint inhibitors

1. Introduction

Tumor formation represents a dynamic and multifactorial process, governed predominantly by genetic and epigenetic alterations within malignant cells and by the structural and functional remodeling of the tumor microenvironment (*TME*) [1]. *TME* refers to the cellular and extracellular environment in which tumors exist [2]. The cellular part is represented by cancerous cells and tumor stromal cells (fibroblasts, endothelial cells, and immune cells). The extracellular part comprises blood vessels, collagen molecules, fibronectin, hyaluronan, and laminin, among others [1].

The essence of *TME* is represented by tumor cells. Notably, tumor cells were first described by Thomas Ashworth in 1869, who observed cells in the bloodstream resembling those from the primary tumor and suggested their role in cancer dissemination. This type of cell has the ability to control the entire spectrum of functions, both cellular and noncellular parts. This happens through complex signaling networks to use the nonmalignant cells to their benefit [1]. It is well known that the nonmalignant cells play a pro-tumorigenic role in all phases of cancer development,

progression, and metastasis [3]. All the interactions present in the *TME* are controlled by a vast bidirectional network (cancer cell-normal cell and vice versa) made up of growth factors, chemokines, cytokines, matrix remodeling enzymes, inflammatory mediators, and novel and exiting mechanisms (circulating tumor cells, exosomes, cell-free DNA, apoptotic bodies) [1].

1.1 Tumor endothelial cells

The endothelium represents a complex network of endothelial cells (*ECs*) that form the inner layer of the blood and lymphatic vessels. This layer is crucial for leukocytes' trafficking between the vessel and the tissue. Also, it is involved in most of the major pathological conditions, from diabetes, cardiovascular disease, and high blood pressure to cancer [4]. Normally, the endothelium has several functions, irrespective of its origin: regulates the passage of oxygen, nutrients, and solutes from vessels to different tissues; regulates the blood flow by maintaining a nonthrombogenic surface; and controls the passage of leukocytes in and out of the tissue [4].

Tumors have different mechanisms of disrupting the normal vascular flow of the organism: neoplastic angiogenesis or vasculogenesis, vessel co-option, and intussusception [5]. *Vasculogenesis* is the process by which new blood vessels are formed from progenitors or stem cells during embryonic development. In contrast, *angiogenesis* involves the generation of new blood vessels from preexisting vessels. It plays a crucial role in both physiological processes, such as tissue repair, and pathological conditions, including tumor progression. While vasculogenesis is fundamental in the early stages of vascular development, angiogenesis is an adaptive response to factors such as hypoxia and inflammation, facilitating the expansion of the vasculature in response to changing tissue demands [4].

Moreover, angiogenesis and vasculogenesis are two processes that depend on circulating signals from chemokines and growth factors, mainly vascular endothelial growth factors (*VEGFs*). Depending on the presence of *VEGF*, different elements evolve. For example, *VEGF-B* is responsible for hematopoiesis and arteriogenesis, while *VEGF-A* sustains angiogenesis [5].

Vascular co-option represents the process by which the tumor cells surround the host vessel and incorporate it within the tumor mass. This process occurs in highly vascularized tissues, such as the brain, lungs, and liver. There is a consensus, explained by Ronca and his team [6], that underlines the fact that the process of co-option is more likely to appear in highly vascularized tissue, which is not included in the highly angiogenic tissue category. Because of that, these cancerous tissues will not respond to anti-angiogenic therapies [5].

Finally, intussusceptive angiogenesis is an alternative mechanism for forming new vessels. During this process, tissue pillars are formed from the capillary wall and extend into the lumen, splitting the vessel on the long axis—the blood flow remains intact [7].

One of the most important cells of *TME* is the *EC*. This type of cell plays a role in the angiogenesis process, tumor development, metastasis, and drug resistance [4]. In cancer, the *ECs* make up the inner layer of the vessels that feed the tumor. Tumoral endothelial cells (*TECs*) have some characteristics that need to be underlined in order to better understand their role: they maintain their ontological endothelial origin, are not immortal, and become morphologically and phenotypically dysregulated at the molecular and cellular level, becoming like the tumor itself [5].

In order for *TECs* to abnormally create vessels inside the *TME*, they need to be stimulated by a series of factors besides *VEGF*, such as chemokines. The *TME*-derived

chemokines can induce vascular permeability and tumor cell extravasation, which leads to tumor cell colonization in a distant area [8]. Also, they can promote recruitment, migration, and proliferation of endothelial cells. These molecules can be targeted, and the new-vessel-formation stimuli can be stopped [8]. There are four chemokine categories: cysteine-cysteine chemokines/cysteine-X-cysteine chemokines/cysteine chemokines/cysteine-X-X-X-cysteine chemokines (CC/CXC/XC and CX₃C). Chemokines' receptors can be used as novel targets for antineoplastic therapies. For example, *CXCR7* (*ACKR3*) is upregulated in various types of cancer, including lung, prostate, pancreatic, and brain. The presence of this receptor on the tumor cell can provide a real advantage that promotes metastasis. Another well-known receptor, *CXCR4*, is highly present in hepatocellular carcinoma (*HCC*). Its inhibition can lead to reduced *VEGF* secretion, which leads to reduced neovascularization and growth at the tumor level. *CCR2* enables vascular permeability and tumor cell extravasation in colon carcinoma in mice models [8].

Pericytes can be found linked with *TECs* within the *TME*. Pericytes are multifunctional cells that surround the surface of *ECs*. These cells are involved in the remodeling of the basement membrane during angiogenesis and tumorigenesis [1]. Also, they exhibit immune functions—attracting leukocytes to exit the blood flow, regulating lymphocyte activation, and eliciting phagocytic activity [9]. Due to their role in the vessels' formation process, they have been proposed as targets for new anti-angiogenic therapies. However, studies have yet to reach a positive consensus in this regard [1].

In conclusion, *TECs* play a crucial role in sustaining tumor vascularization, contributing to cancer development and further progression. Targeting signaling pathways, such as *VEGFs* and chemokine receptors expressed on their surface, represents a promising therapeutic approach, potentially disrupting tumor angiogenesis and limiting disease advancement. There are several advantages in targeting *TECs* to slow down tumor growth. First, *TECs* are *accessible* to therapeutic agents present in the circulation. Second, *TECs* are not immortal, so there is a minimal probability for them to develop drug resistance compared to cancerous cells. Third, Aird proposed that the inhibition of a single *TEC* could impair the function of at least 100 neighboring *TECs* [10]. Further work and commitment are needed in order to develop novel drugs that suppress *TECs*' function inside *TME*.

1.2 Circulating tumor cells

The progression of cancer from its primary site to a secondary one is a complex process, and also the leading cause of cancer-related deaths. The principal factor behind this process is the circulating tumor cell. A circulating tumor cell (*CTC*) detaches itself from the primary tumor site and spreads through the bloodstream to colonize other tissues and organs [11].

The initial mention of this cell was made in 1869 by Ashworth, who divided these cells into three main categories: epithelial, mesenchymal, and hybrid. Even though most of the *CTCs* are destroyed by the immune system, a small percentage of *CTCs* can evade immune clearance, leading to tumor recurrence or metastasis. However, the successful establishment of a metastatic site requires overcoming several obstacles [11]:

1. Adhesion: surrounding cells and *ECM* of the primary tumor are both strongly adhered to one another. *CTCs* must overcome these adhesion forces to enter the blood/lymphatic system.

2. Invasion: *CTCs* must be able to invade the surrounding tissues so they can reach the vessel and further disseminate.
3. Shear stress: once entered into the bloodstream or lymphatic system, *CTCs* are subjected to shear stress. This can lead to mechanical damage to the cells and their apoptosis.
4. Immune system surveillance: *CTCs* need to be able to avoid being detected by the immune system in order to survive and form secondary tumor sites.

Being directly detached from the primary lesions, these cells precisely represent the tumor. By doing so, they are able to transmit the information needed for their detection, as well as for tumor diagnosis, follow-up, and treatment [1]. Thus, they represent vital tools for prognosis and diagnosis. *CTCs* can be found in a wide range of cancers using a comprehensive biomarkers agenda (epithelial, mesenchymal, stemness, and specific markers—including immune checkpoints). The most important aspect of *CTCs* is the presence of *CD274* and *CD47* on the *CTC*'s surface. By using dual blockade of *CD274* (PD-L1/B7-H1) and *CD47* with monoclonal antibodies, we can block both the “no find me” and “don't eat me” signals [12].

There are also studies regarding other dual blockades based on *CTCs*' markers: epithelial cell adhesion molecule (*EpcAM*) and human epidermal growth factor receptor 2 (*HER2*). Dong et al. [13] reported that this blockade, which is based on a dual double-stranded aptamer ring conjugate, can bind two distinct biomarkers simultaneously and induce the apoptosis of the presenting cell, thus inhibiting tumor progression.

Because of their importance in the diagnosis and prognostic steps, there is an intense need for creating the perfect method of detection. There are methods based on the cell morphology, especially diameter, and immunological status—by using immunomagnetic, real-time *PCR* (a highly sensitive method), cytometry-based methods, and microscopic methods—next generation of liquid biopsy [14]. Liquid biopsy represents the new and existing method of detection due to its rapidity and noninvasiveness in the tissue-obtaining process.

1.3 Extracellular matrix

Extracellular matrix (*ECM*) is a key component of the *TME*. It represents the scaffold of all tissues and organs through the production of macromolecules. The most important macromolecules are glycoproteins, glycosaminoglycans (such as hyaluronic acid), proteoglycans (such as heparan sulfate), collagens, laminins, and enzymes [1]. Their purpose is to support the biomechanical activities and functions in the body [2]. It is important to keep in mind that *ECM* is an *active* component of the *TME*, being composed of active tissue elements that influence cell adhesion, proliferation, communication, and cancer cell migration by altering their physical properties, composition, and topography [15].

The *ECM* is, in fact, the fundamental requirement for creating an adherable environment that supports cell survival and proliferation [16]. We can describe it from three main perspectives: *molecular*, *structural*, and *functional*.

From a molecular point of view, the *ECM* encompasses structural proteins, such as collagen and elastin; adhesion proteins, such as fibronectin and laminin; glycosaminoglycans; and proteoglycans [16]. In terms of structure, *ECM* contains the basement membrane and interstitial connective tissue matrix [16].

Finally, from a functional perspective, the *ECM* sustains cell proliferation and differentiation by maintaining tissue homeostasis [17]. Another important function of the *ECM* is represented by the possibility to influence drug delivery—it *acts like a barrier* by increasing/decreasing its stiffness and by facilitating metastasis *by forming a permissive/restrictive gate for various soluble factors/CTCs/exosomes detached from the primary tumoral site* [18].

For a better understanding of the *ECM*'s complexity, there is a new concept designed by Naba and colleagues—the *matrisome* [19]. This developing technique was screening core *ECM* elements through bioinformatic approaches and describing the proteins related to *ECM* but not included in the *ECM*'s core components. As a summary of their research objective, they aimed to divide the genetic components of the *ECM* into *core matrisome genes* and *matrisome-associated genes*, which either interact with or remodel the *ECM*. The core matrisome genes include glycoproteins, collagens, and proteoglycans, while matrisome-associated genes incorporate *EMC*-related proteins, different *ECM* regulators, and various soluble factors [20].

Collagen is the main component of the *ECM*. It is commonly seen in all tumor tissues and is accountable for the *ECM*'s stiffness [21]. Collagen alterations have been reported in various types of cancer. For example: *COL6A3* and *COL12A1* genes are mutated in breast cancer; *COL7A1* and *COL11A1* are mutated in stomach cancer, while *COL3A1* is highly mutated in liver cancer [20]. Another core matrisome gene dysregulated within *TME* is represented by the glycoprotein family. Some of them are upregulated in cancer—laminin in pancreatic cancer [22]; others are downregulated—tenascin family member (*TNXB*)—in breast, lung, and pancreatic cancer [23]. Matrisome-associated genes are represented by *ECM*-affiliated proteins—proteins of the annexin and galectin family, secreted factors—filaggrin (*FLG*) *S10* family (overexpressed in pancreatic and colon cancer), and *ECM* regulators—lysyl oxidase family [21].

Attention is drawn toward the possibility of creating new biomarkers—both for diagnosis and prognosis—based on *ECM* components. There are several studies that revealed various groups of matrisome genes that exhibit nearly perfect accuracy for diagnosing lung cancer—for example: 29 matrisome genes or 28 matrisome gene groups used for detecting non-small cell lung cancer (*NSCLC*) [21].

Collagen has demonstrated considerable potential as a prognostic factor across multiple cancers. In ovarian cancer, elevated expression of *COL5A2* is linked with decreased progression-free survival (*PFS*), while in stomach cancer, it is associated with improved overall survival (*OS*) [21]. Also, there is incipient research that shows promising results regarding the connection between matrisome genes and therapy success. For example, the overexpression of *COL3A1*, *COL10A1*, and *COL11A1* in ovarian cancer is associated with chemotherapy resistance, while the presence of mutated *COL5A2* in stomach cancer is linked with improved response to pembrolizumab [21].

1.4 Other elements of tumor microenvironment

Besides *CTCs*, *ECM*, and *TECs*, the *TME* has numerous components. These components will be further discussed in this section of the chapter.

1.4.1 Cancer-associated fibroblasts (CAFs)

CAFs constitute key components of the *TME*, serving as the principal source of *ECM* production. Beyond their structural role, *CAFs* actively contribute to

tumorigenesis by inhibiting apoptosis and facilitating cancer cell survival, proliferation, and migratory capacity. CAFs also play a part during angiogenesis by producing fibroblast growth factor 2 (*FGF2*) and vascular endothelial growth factor (*VEGFA*) in different types of cancers [24].

1.4.2 Circulating free-DNA (*cfDNA*)

cfDNA develops from both living and necrotic cells [25]. It consists of short (<200 bp) double-stranded DNA fragments obtained at very low concentrations [1]. During cancerous conditions, *cfDNA* emerges not only from cancer cells but also from *TME* components and nonmalignant cells. Cancerous conditions are frequently characterized by elevated levels of circulating cell-free DNA (*cfDNA*)—hence why it can offer diagnostic evidence. What is impressive about *cfDNA* is that it can indicate genetic and epigenetic information about a derived tumor, with noninvasive means merely from the plasma and serum of the patient [1]. This practice can eliminate the need for invasive biopsy. Since it contains this form of information, *cfDNA* is currently considered a highly important tool for the personalization of oncologic treatment as well as for predicting the course of the disease through its quantitative measurement.

1.4.3 Extracellular vesicles (*EV*)

Extracellular vesicles, encompassing both *exosomes* and apoptotic bodies, play a fundamental role in the dynamic crosstalk within the *TME*. Exosomes are small vesicles

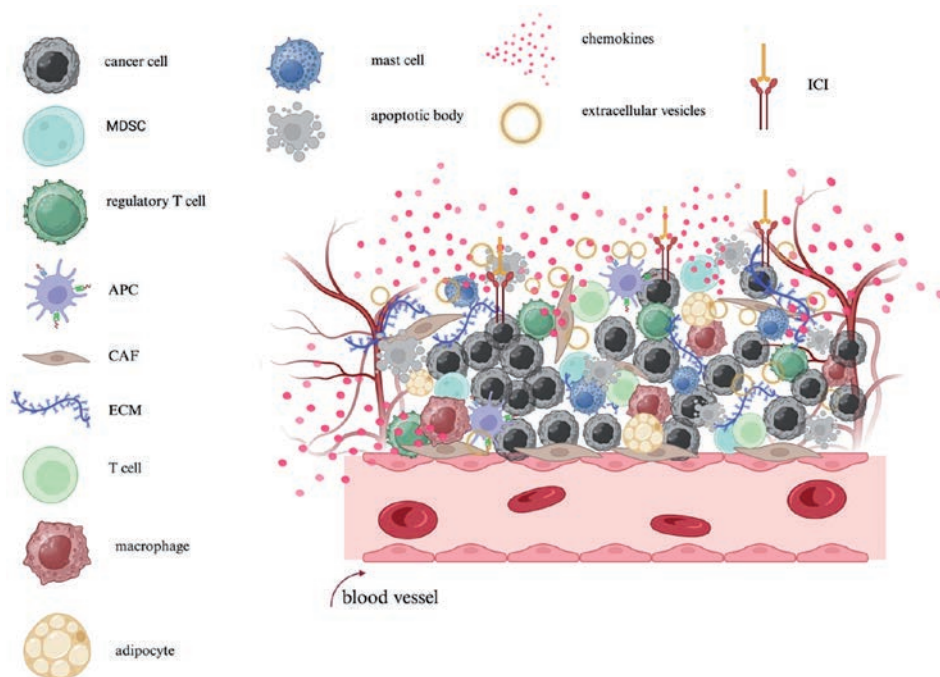


Figure 1. Visual representation of *TME* and its elements. Abbreviations: myeloid-derived suppressor cell (MDSC); antigen presenting cell (APC); cancer-associated fibroblast (CAF); extracellular matrix (ECM); immune checkpoint inhibitor (ICI).

(30–150 nm) originating from the endosomal pathway. Apoptotic bodies, larger vesicles (500–2000 nm), are released during the final stages of apoptosis [26]. Together, they act as vehicles for intercellular communication. They also transport a wide array of biomolecules—proteins, lipids, mRNAs, microRNAs, and even DNA fragments [1]. This can profoundly influence the phenotype and function of recipient cells.

In the *TME*, tumor-derived EVs are instrumental in promoting immune evasion, supporting angiogenesis, inducing therapy resistance, and facilitating metastasis by modulating the behavior of immune cells (e.g., T cells, macrophages, dendritic cells), fibroblasts, and endothelial cells. Notably, EVs can suppress cytotoxic immune responses or promote immunosuppressive phenotypes (such as M2-like macrophages), thereby diminishing the efficacy of immunotherapies [27]. Given these multifaceted roles, EVs not only reflect the molecular landscape of the tumor but also represent promising targets for therapeutic intervention and biomarkers for predicting response to immunotherapy and targeted therapy strategies (**Figure 1**) [28].

2. The therapeutic approaches based on tumor microenvironment components

Cancer is not a solitary disease. Instead, it represents a complex production—tumor cells and non-tumor cells. The non-tumor cells play an important role in supporting the development of the tumor cells. These components are represented by the immune cells, fibroblasts, endothelial cells, and adipocytes. Each of them has a well-defined role within *TME* [1].

The therapeutic approaches based on *TME* components have their foundation in these cells, their cell-cell interactions, and their extracellular support—*ECM*. Based on these elements, complex therapeutic protocols can be developed. These new protocols can be considered superior to classical protocols such as chemotherapy, radiotherapy, and surgery. The primary advantage of targeted therapy lies in its ability to spare normal, non-tumoral cells, which are significantly more genetically stable than tumor cells [29].

The following section discusses the therapeutic approaches based on *TME* components, including cells, *ECM*, facilitators of cell-cell interactions, and vessel-formation processes.

Inside *TME*, there is an active contradictory interaction between anti-tumoral functions and pro-tumoral functions of each component. The anti-tumoral functions are regulated by immune cells, macrophages, and *normal* fibroblasts. On the other hand, the pro-tumoral functions are mainly mediated by immune cells. Among them, the most important are myeloid-derived suppressor cells, M2-tumor-associated macrophages (*TAMs*), regulatory T cells (*Tregs*), cancer-associated fibroblasts (*CAFs*), cancer-associated adipocytes (*CAAs*), and finally, tumor-associated endothelial cells (*TECs*) [30].

Cancer cells inherently display a set of hallmark features that form the basis of oncologic therapeutic strategies, such as uncontrolled proliferation, cellular immortality, the exploitation of surrounding cellular resources, and the disruption of tissue homeostasis. Consequently, therapeutic interventions are designed to counteract these pathological traits [30].

2.1 Reciprocal cell-cell interaction

Tumor progression or regression can be influenced by various cell-cell interactions inside the *TME*, including *tumor cell-non-tumor cell interactions*. In a normal tissue,

these connections are mediated through receptors/*ECM* constituents/*ECM* enzymes or diverse soluble mediators: growth factors, chemokines, and cytokines [31]. By extrapolation, any structural or functional alteration of these elements may lead to the onset of cancer and promote its progression by preventing the reestablishment of tissue homeostasis and hindering pharmacological intervention. The following section of this chapter will provide a concise overview of the cellular interactions within the *TME*, aiming to underscore the intricate molecular architecture and dynamic complexity that characterize this specialized niche.

Starting with the immune cells, the cellular interaction between them and cancer cells can be explained by the following three main phases [30]. Each phase has its own mediators and interactors that determine either tumor progression or suppression:

- **Elimination phase:** immune cells function optimally and are able to destroy tumor cells. The elimination process involves the participation of both the innate and adaptive branches of the immune system [30]. During the elimination phase, tumor cells are recognized by immune cells, such as T cells and macrophages, through the expression of surface molecules, including ligands for the *NKG2D* receptor.
- **Equilibrium phase:** those tumoral cells that had survived the elimination phase now enter into an equilibrium with the environmental immune cells. This phase can last many years—the *tumoral cells enter into a dormant phase*. The T cells are the main immune cells involved, and they preserve the communication between immune cells and tumoral ones. During these interactions, some soluble factors, such as Interferon (*IFN*) and Tumor necrosis factor (*TNF*), are also involved. Moreover, *IFN* was found to be the key player in the progression from the elimination to the equilibrium phase.
- **Escape phase:** those cells that were dormant during the equilibrium phase can be reactivated and enter the escape phase. At this point, tumoral cells have various mechanisms to avoid immunosurveillance: they produce immunosuppressive cytokines—interleukin 10 (*IL-10*)—or they can recruit cells with immunosuppressive properties—*Tregs*.

Building upon the previously outlined framework of the three molecular interactions “E’s,” it is also important to consider the complex network of cellular interactions within the *TME*. Beyond molecular signaling, cancer cells engage in dynamic crosstalk with other cellular components, including *CAFs*, *TECs*, *TAMs*, and adipocytes. Each of these interactions is mediated by a range of soluble factors that contribute to the functional and structural remodeling of the *TME*. *CAFs*’ secreted factors represent the drivers of their pro-tumorigenic functions: chemokines/growth factors/*ECM* proteins. These molecules can promote tumor cell survival, proliferation, and metastasis. *CAFs* are also involved in new vessel formation through the production of *VEGF*. They also participate in tumoral inflammation through the production of interleukin 1/6 (*IL-1/IL-6*) and *TNF* α [30].

That is why the metastasis process is influenced by the direct, physical contact between *CAFs* and cancerous cells—contact mediated by the junction between two cadherins: E-cadherin expressed on tumor cells and N-cadherin on the *CAFs* [30]. With regard to the viability of tumor cells, it is well established that cancer cells need oxygen and various nutrients for their survival and further development.

In order to survive, tumors can obtain their vascularization in two ways: creating new vessels—*angiogenesis*—or using preexisting vessels for their own nutrition—*vascular co-option*. The reciprocal interaction between these cells is mediated by several factors secreted by cancer cells: *VEGF* and platelet-derived growth factor (*PDGF*). Together, they stimulate endothelial cell proliferation and new vessel formation.

Regarding macrophages and their role within the *TME*, it is important to recognize that *TAMs* are normally divided into M1 pro-inflammatory and M2 anti-inflammatory macrophages. *TAMs* also express *PD-L1* and *CD47*, important ligands for the immune cells. Also, they promote vascular formation, both blood vessels and lymphatic, by secreting various factors such as *VEGF-1/VEGF-C/VEGF-D/FGF* [30]. This cell type exhibits significant potential in the context of targeted therapies.

A less extensively studied cell type in the context of potential targets for antineoplastic therapies is the adipocyte, whose role within the *TME* is gaining attention. A defining characteristic of cancer is the elevated use of lipids. In various cancer types, the adipocytes are present in the *TME* in different quantities. The crosstalk between adipocytes and tumoral cells can be physical or through secreted factors, and the main goal is to induce lipolysis as a source of lipids—energy for tumor cell proliferation and invasiveness [30].

2.2 Therapeutic strategies for modulating the tumor microenvironment to improve treatment efficacy

Currently, there are five *manipulating* strategies that can be applied to switch the way of working of the *TME*—from *pro-cancerous* to *anti-cancerous*. This includes [32]:

- Enhancing T cell infiltration into immunologically “cold” tumors
- diminishing the abundance of myeloid-derived cells
- suppressing regulatory T cell (*Treg*) function
- augmenting T cell-mediated cytotoxic activity
- inducing further tumor-associated genetic alterations

It is important to underline that these strategies are not curative. To be more exact, these can be used in combination with chemotherapy/immunotherapy in order to enhance their antitumoral effect.

In summary, the five strategies above refer to the potential therapeutic targeting of key components within the *TME* [32]. Facilitating *T cell migration* into the *TME* contributes to the conversion of “cold” tumors, characterized by an absence of T cells, into “hot” tumors. This migration is mediated by specific molecular interactions, such as those between *CXCR4* and *CXCL12*. Reduction of the myeloid cell population within the *TME*—cells known to inhibit T cell function—can be achieved through the modulation of activation pathways including *JAK*, *BTk* (expressed on B cells), and *PI3K*, or by using *tyrosine kinase inhibitors*, which shift macrophage polarization from the immunosuppressive M2 phenotype to the pro-inflammatory M1 phenotype. Regulatory T cell (*Treg*) depletion is accomplished by targeting the surface marker *CD25*. Enhancing T cell functionality involves the use of soluble factors such as *interleukin-15*. Ongoing studies are investigating the development of therapeutic agents

that combine immunotherapy with interleukin-15 (IL-15) activity. For instance, IL-15 is linked to an anti-PD-1 antibody [33]. Finally, inducing *additional genetic mutations* within tumor cells is considered a high-risk but potentially effective strategy, as it may lead to the generation of neoantigens that can be specifically targeted by the immune system. This can be achieved through the use of DNA-damaging agents such as *temozolomide* or by employing *inhibitors of DNA repair mechanisms* [32].

2.2.1 Targeting cells and extracellular matrix: Different therapeutic approaches

Targeting drugs represents a promising therapeutic approach compared with chemotherapy. The principal advantage of targeted drugs over chemotherapy is that the exposure of healthy cells is reduced—enhanced concentration at the tumor site only [34]. Targeting the *TME* may result in a higher treatment efficacy by affecting both tumor cells and tumor-supportive cells—*CAFs*, macrophages, endothelial cells, etc. We can actively target these molecules by internalizing them into the receptor cell by using specific ligands and antibodies, *or* we can passively target them using the properties of newly formed tumoral vessels. They can be considered as being leaky vessels with high permeability and decreased lymphatic drainage. This leads to an increased retention of various products, in our case, drug molecules [34].

Of course, there are some *limitations* regarding the use of targeting strategies: first, tumors are very heterogeneous media. By using targeting agents, we will remove just one subset of cells—those expressing that target. Second, targeted strategies may miss the entire spectrum of supportive tumor cells. Thirdly, the delivery system to the target is extremely complex, especially after internalization into the cell, due to the action of lysosomal compartments, which entrap the drug delivery system, thus preventing reaching the site of action, for example, the nucleus [34].

In this section, we will briefly discuss the strategies aimed at overcoming the second limitation. As discussed up until now, tumoral *TME* differs from the normal *TME*. It has a heterogeneous composition, each element representing a potential target.

For example, *tenascin-C*, a glycoprotein, is highly expressed in the *TME* of various solid tumors, including lung, breast, colon, and ovarian. This molecule can promote *angiogenesis, tumor proliferation, and metastasis* [35]. There are several studies that suggest the use of tenascin-C as a potential target for therapies. In this regard, Lin et al. [36] described a tenascin-C strategy by using *doxorubicin-loaded liposomes* that will bind tenascin-C with the aim of sulfatide-expressed receptors on the surface of the liposomes. In mice bearing subcutaneous gliomas and colorectal carcinomas, it was shown that overall survival is prolonged and the spectrum of side effects is reduced [34, 36, 37]. Another molecule from *TME*, *collagen*, can also be used as a target. Liang and his team [38] designed an antibody conjugate by combining the collagen with the Fab fragment of an antibody—*cetuximab* directed against *EGFR*.

Another therapy choice is represented by the well-known anti-angiogenic therapy, which aims to inhibit the formation of new blood vessels that sustain tumor growth [39]. Agents such as *bevacizumab* (anti-VEGF antibody), *sunitinib*, and *sorafenib* (multi-kinase inhibitors targeting *VEGFR* and platelet-derived growth factor receptor (*PDGFR*)) are already in clinical use for various solid tumors. Other investigational compounds, such as *Tasquinimod*, have entered phase III trials for castration-resistant prostate cancer (*CRPC*), targeting both angiogenesis and immunomodulatory pathways. These therapies not only restrict the tumor's vascular system but also aim to normalize aberrant vasculature to improve immune cell infiltration and drug delivery [39].

Simultaneously, CAFs are being explored as therapeutic targets due to their strong involvement in extracellular matrix remodeling and immunosuppressive signaling. Several therapeutic approaches aimed at modulating or eliminating CAFs are under investigation. One of the most studied targets is fibroblast activation protein (FAP), which is highly expressed on CAFs but minimally present in normal tissues. FAP-targeted agents, including monoclonal antibodies (e.g., Sibrotuzumab) [40] and FAP-specific CAR-T cells, have entered clinical trials. Although early trials such as those with Sibrotuzumab showed limited efficacy as monotherapy, combination approaches with immune checkpoint inhibitors are currently being explored.

Another major axis of intervention involves TGF- β signaling, a key pathway in CAF activation and ECM deposition. Inhibitors such as Galunisertib (a TGF- β RI kinase inhibitor) have demonstrated immune-modulatory effects and are being tested in combination with other immunotherapies, particularly in pancreatic and hepatocellular carcinoma [40]. Additionally, NOX4 inhibitors (e.g., GKT137831), which block reactive oxygen species (ROS) production in activated fibroblasts, have shown great potential in preclinical models by reversing the myofibroblastic CAF phenotype and enhancing immune infiltration [40].

An alternative strategy is not to eliminate CAFs but to reprogram their phenotype. Metabolic modulators like Metformin have been shown to suppress the tumor-supportive functions of CAFs, promoting a shift toward a quiescent state [41]. Similarly, vitamin D analogs, such as calcipotriol, have been reported to revert CAFs toward a less activated phenotype, improving the response to chemotherapy in pancreatic cancer models. Inhibitors such as DRP-104 (glutamine antagonist), CB-839 (glutaminase inhibitor), and INCB001158 (arginase inhibitor) have shown promise in early-phase trials, often in combination with immune checkpoint inhibitors [42]. These agents target critical metabolic dependencies of the tumor and of the stromal cells, potentially restoring immune functionality and improving treatment responsiveness. While the clinical translation of CAF-targeted therapies is still in early stages, the heterogeneity of CAF populations—ranging from immunosuppressive to pro-inflammatory subtypes—needs a more refined approach. Future strategies may involve selectively targeting pro-tumorigenic CAF subtypes while preserving or enhancing the anti-tumorigenic ones. As more insights into CAF plasticity and function emerge, these therapies hold promise for overcoming resistance mechanisms and improving outcomes when integrated with existing immuno- and targeted therapies [42].

Collectively, these approaches reflect a paradigm shift in cancer therapy—from targeting tumor cells alone to modulating their supportive microenvironment. By disrupting angiogenic signaling, reprogramming fibroblasts, and altering tumor metabolism, these therapies may improve clinical outcomes and overcome resistance mechanisms that limit the efficacy of conventional treatments.

Other molecules that can be used as potential targets are represented by galectin (cisplatin payload), heparan sulfate (paclitaxel payload), and chondroitin sulfate (cisplatin/doxorubicin payload) [34].

2.2.2 Immunotherapy based on tumor microenvironment: Present and future

Over the last years, cancer has become more and more treatable with the development of *immunotherapy*. This type of therapy works around the following idea: it blocks the immune system by using molecules—*antibodies* directed against various ligands/receptors expressed on cell surfaces [43].

Monoclonal antibodies (*mAbs*) have become indispensable agents in contemporary oncology, providing highly specific and multifaceted therapeutic strategies. Since their initial development in the 1970s [44] and subsequent implementation in cancer diagnosis and treatment, *mAbs* have demonstrated significant clinical utility in targeting both tumor cells and components of the *TME* [45]. These antibodies primarily function by recognizing tumor-associated antigens expressed on the surface of malignant cells, thereby enhancing immune-mediated cytotoxicity, particularly via mechanisms such as antibody-dependent cell-mediated cytotoxicity (*ADCC*) [46]. Additionally, *mAbs* can inhibit key oncogenic signaling pathways that promote tumor cell proliferation and survival. The conjugation of *mAbs* with cytotoxic agents or radioactive isotopes further enables precise delivery of therapeutics, thereby improving efficacy while limiting off-target effects. Recent advances include bispecific antibodies capable of simultaneously engaging T cells and tumor cells, effectively bridging and activating immune effector mechanisms. Furthermore, therapeutic antibodies that target immune checkpoints or angiogenic pathways modulate the tumor microenvironment to restore anti-tumor immune responses and suppress tumor progression [45]. Collectively, these mechanisms underscore the central role of monoclonal antibodies in the evolving paradigm of precision cancer therapy.

The main targets are represented by *CTLA-4* and *PD-1/PD-L1* [47]. However, in the last years, new promising checkpoint molecules have been investigated more deeply. For example, lymphocyte-activation gene-3 (*LAG-3*), T-cell immunoglobulin 3 (*TIM-3*), T-cell immunoglobulin, and ITIM domain (*TIGIT*) [47]. *LAG-3* is a type I transmembrane protein, mainly expressed on T cells, natural killer cells, B cells, and dendritic cells. It is involved in the transduction of inhibitory signals regarding immune cells [48]. There are several drugs available for this molecule, including relatlimab. There is strong evidence regarding the efficacy of this type of therapy, especially when it is combined with anti-PD-1 therapy. RELATIVITY-047 trial, a double-blind, randomized trial, suggested that this dual therapy can enhance the immune response and improve the outcome of patients diagnosed with metastatic or unresectable melanoma [49].

Regarding *TIM-3* and its ligand, galectin-9 (*GAL-9*), it shows potential in apoptosis regulation and T cell immune tolerance. By blocking *TIM-3/Gal-9* pathway, it is possible to evade T cell depletion and enhance treatment response and reduce drug resistance [50]. For *TIGIT* there are several clinical studies conducted mainly in China: MK-7684A-003 trial (PD-L1 $\geq$ 1%, MK-7684 + Pembrolizumab, NCT04738487) or AdvanTIG-302 trial (PD-L1 $\geq$ 50%, BGB-A1217 + Teicilizumab, NCT04746924) that show promising results regarding the usage of *TIGIT* as a therapeutic target.

Understanding the tumor immune microenvironment represents the key to developing and using the right therapy for our patients. In cancer, *TME* has an abnormal composition. Each cell and soluble factor represents a potential weapon for transforming normal elements into malignant ones, hence increasing the tumor's aggressiveness in the body. Before applying a specific immunotherapy, it is important to know which immunological tumor subtype we are dealing with. From an immunological point of view, there are four types of tumors [47]:

- Hot tumors: elevated T cell concentration.
- Altered-excluded tumors: T cell contractions are higher at the periphery of the tumoral area, but there is no tumoral infiltration.

- Altered-immunosuppressed tumors: there is tumoral infiltration with T cell immunosuppressive characteristics.
- Cold tumors: lack of T cells.

As it was previously presented, the main cells of *TME* are represented by: *TAMs* with the dual phenotype potential, M1 and M2; endothelial cells; dendritic cells (*DCs*); Neutrophils- N1 and N2 phenotype; *NKs*; and T cells. A critical part of the immune system is represented by the T cell, which can be divided into three main categories with different functions and markers on their surface: cytotoxic T cells (*CD8⁺*), helper T cells (*CD4⁺*), and regulatory T cells (*CD4⁺*).

CD8⁺ cells are the primary tumor-killing cells. Their elevated level correlates with a better response to immunotherapy and an overall better prognosis for patients. Even so, *TMEs* have the capacity to modify the functionality of these cells through five mechanisms (*senescence, tolerance, anergy, exhaustion, and ignorance*) [51]. These mechanisms are strongly linked with metabolic changes—*glucose, lipids, and hypoxia*—and molecular rearrangements of T cells. It was stated before that the accumulation of *lactate and cholesterol* is linked with a reduction of infiltrating T cells into the tumor and with an upregulation of several checkpoints, including *PD-1* and lymphocyte-activation gene 3 (*LAG-3*), that will lead, eventually, to T cell exhaustion [52]. *CD4⁺* helper cells can be differentiated into three subsets: T helper cells 1/2/17 (*Th1, Th2, and Th17*). Each plays a distinct role. *Th1* appears under the action of *IL-12* and *INF- γ* and has an antitumoral effect overall. On the other hand, *Th2* cells promote tumor progression by secreting cytokines such as *IL-4, IL-5, IL-10, and IL-17*. The role of *Th17*-secreted cytokines still remains unclear [53]. Regulatory *CD⁺* cells, *Tregs*, express a high affinity for *IL-2's* receptor and secrete a variety of cytokines: *TGF- β , IL-10, and IL-35*. An important aspect to remember about this type of cell (*CD8⁺*) is that its viability is closely linked to fatty acid metabolism, unlike other T cell types, which primarily rely on glucose and the hypoxic environment. Therefore, the production of these cells can be reduced by targeting the enzymes involved in fatty acid oxidation [52].

Another important aspect in understanding the use and benefits of immunotherapy is the exploration of the signaling pathways within the *TME* and their applicability in drug development. The *TME* is influenced by several immune signaling pathways that play crucial roles in tumor surveillance and immune response modulation. Among the most important is the *STING (Stimulator of Interferon Genes)* pathway, which is activated by cytosolic *DNA* and leads to the production of type I interferons, thus fostering anti-tumor immunity [47].

STING agonists, such as *DNX-2401* and *MIW815*, have shown promise in preclinical models as immune-enhancing agents. Additionally, *Toll-like receptors (TLRs)*, which recognize pathogen-associated molecular patterns (*PAMPs*) and danger-associated molecular patterns (*DAMPs*), activate inflammatory cascades that aid in immune system activation [47].

TLR7/8 agonists, like *Imiquimod* and *Resiquimod*, have been explored as potential adjuvants to stimulate anti-cancer responses. The *NOD-like receptors (NLRs)*, including *NLRP3*, are central to inflammasome activation, which triggers the release of pro-inflammatory cytokines such as *IL-1 β* and *IL-18*. This pathway can support immune activation but may also exacerbate chronic inflammation in the *TME*, as seen with *VX-765*, an *NLRP3* inhibitor, which is being investigated in cancer treatment [47].

Additionally, *RIG-I-like receptors (RLRs)* sense viral RNA and activate type I interferon responses, with *MDA5 agonists* showing potential in stimulating anti-tumor

immunity [47]. These pathways collectively offer valuable therapeutic targets; however, careful modulation is essential to balance efficacy and prevent adverse inflammatory effects.

The therapeutic manipulation of these pathways holds great promise for advancing cancer immunotherapy, as exemplified by ongoing clinical trials aimed at enhancing immune responses while minimizing harmful consequences. There are several clinical trials that highlight the potential benefit of combining classical immune checkpoint inhibitors (ICIs) with novel molecules. For instance, a phase Ib trial showed sustained disease control in over 50% of advanced or metastatic *NSCLC* cases by using a dual therapy—*nivolumab* and *TLR9 agonist DV281* [54]. In this case, tumor regression was observed in both the injected and non-injected areas, including visceral lesions.

Another study was focused on head-and-neck cancer patients [55]. By using both *PD-1* blockade and a *TLR9* agonist, *SD-101*, an objective response rate (ORR) was reached in 24% of cases, with a higher value in *HPV+* patients—4%.

Overall, *ICIs* are now the main therapeutic practice in various cancer types. The primary targets are *CTLA-4* and *PD-1/PD-L1*, which have gained universal utilization over the last years. *CTLA-4* represents a coinhibitory molecule localized on the T cell surface and works as a negative modulator of T cell activation. Its targeting antibody—*ipilimumab* and *tremelimumab*—emerges as a pivotal molecule used in different cancers, such as lung, liver, or melanoma. On the other hand, *PD-L1* promotes immune tolerance by engaging *PD-1* on T cells, leading to suppression of cytokine production and lymphocyte proliferation. It was stated that the inhibition of *PD-1* or *PD-L1* will enhance tumoral regression by rejuvenating the cytotoxic property of T cells. Its specific antibodies—*atezolizumab*, *avelumab*, *nivolumab*, *pembrolizumab*, *cemiplimab*, and *dostarlimab*—are used in different types of cancers, such as lung, liver, kidney, gastrointestinal cancers, or breast, leading to spectacular responses [56].

Comparable to all other types of cancer-related therapies, *ICIs* encounter some limitations and challenges. One of the major ones is that *ICIs*' performance depends on the tumor's immunological status—*cold* or *hot*. Thus, transforming a cold tumor

Drug name	Target	Indicated for particular forms of cancer
Atezolizumab	<i>PD-L1</i>	Breast, liver, lung, sarcoma
Avelumab	<i>PD-L1</i>	Kidney
Cemiplimab	<i>PD-1</i>	Lung
Dostarlimab	<i>PD-1</i>	Endometrial cancer
Durvalumab	<i>PD-L1</i>	Bladder, liver, lung
Ipilimumab	<i>CTLA-4</i>	Lung, liver, melanoma
Nivolumab	<i>PD-1</i>	Bladder, GIs, kidney, melanoma
Pembrolizumab	<i>PD-1</i>	Bladder, breast, cervical, colorectal
Relatlimab	<i>LAG-3</i>	Melanoma (combination with Nivolumab)
Tremelimumab	<i>CTLA-4</i>	Liver, lung

Abbreviations: cytotoxic T-lymphocyte-associated protein 4 (*CTLA-4*); lymphocyte-activation gene 3 (*LAG-3*); programmed cell death protein 1 (*PD-1*); programmed death-ligand 1 (*PD-L1*).

Table 1.
Types of checkpoint inhibitors and their clinical application.

into a hot one still represents a major need in cancer treatment. Another challenge is related to the appearance of side effects, mainly adverse *immune* responses. Immune-related adverse events (*irAEs*) encompass a wide spectrum of reactions that affect nearly every organ and system within the body [57]. The severity of these side effects varies between low-grade (one-two grade)—90% of patients—and high-grade (three-four grade)—10% of patients. The onset is variable, in contrast with the toxicities related to chemotherapy or radiotherapy. Some of the *irAEs* manifest within days after the administration; others may appear months later [56]. The conventional strategy for addressing immune-related adverse events (*irAEs*) typically entails the interruption of immune checkpoint blockade therapy and the initiation of high-dose corticosteroid treatment. Irrespective of the specific therapeutic protocol employed, effective *irAE* management critically depends on the prompt identification of clinical manifestations and the implementation of a timely, proactive treatment approach, frequently necessitating coordination among a multidisciplinary team of healthcare specialists (**Table 1**) [58].

3. Conclusion

TME represents a highly dynamic and complex system that has emerged as a pivotal element in cancer progression and treatment development. Its complexity stems from its numerous components, each of them playing distinct roles in maintaining tumor cell viability, promoting their proliferation, and later facilitating the metastasis cascade. Importantly, tumor cells have the ability to manipulate surrounding normal cells to support their growth and progression through various signaling pathways and multiple soluble factors. Recent research has highlighted its therapeutic potential, with several of its components—both cellular and noncellular—proving valuable in developing novel strategies that selectively target malignant cells while sparing healthy cells and tissues. Throughout this chapter, we have reviewed the diverse cellular elements of the *TME*, emphasized the dual role of the *ECM* as both a structural scaffold and a modulator of tumor behavior, and explored the reciprocal cellular interactions that contribute to tumor immune evasion and therapy resistance.

A critical point discussed was the relevance of immunological profiling—distinguishing between “hot” and “cold” tumors—and its implications for selecting appropriate immunotherapeutic approaches. Understanding these immune landscapes is fundamental to optimizing treatment outcomes and enhancing patient response rates.

Looking ahead, several directions in oncology emerge with promise. The molecular and metabolic elements of the *TME*—especially those less explored, such as *ECM* subtypes and the metabolic niche—warrant further investigation. Ongoing studies are exploring how metabolic pathways, including aerobic glycolysis and glutamine metabolism, influence tumor dynamics and how external factors such as diet, chronic stress, lifestyle, and environmental exposure may modulate therapeutic efficacy, response, and disease progression.

CAFs, in particular, have shown considerable potential as therapeutic targets. Accumulating functional and correlative analyses suggest that improved treatment outcomes could be achieved by disrupting their pro-tumorigenic roles. Similarly, a better understanding of the *TME* at the molecular level—through the identification of new targets and recharacterization of known ones—could dramatically expand the repertoire of available cancer therapies.

In summary, the *TME* holds great potential as a source of therapeutic targets. Continued research combining molecular characterization, clinical correlation, and drug development will be essential in translating these insights into effective and individualized cancer treatments.

Conflict of interest

The authors declare no conflict of interest.

Author details

Anca-Florina Zgura^{1,2*}, Rodica-Maricela Anghel^{1,2} and Stefania Chipuc²

1 Faculty of General Medicine, University of Medicine and Pharmacy “Carol Davila” Bucharest, Bucharest, Romania

2 Oncology Institute “Prof. Dr. Alexandru Trestioreanu,” sos. Fundeni, Bucharest, Romania

*Address all correspondence to: medicanca@gmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Eynali S, Baradaran B. Tumor microenvironment complexity and therapeutic implications at a glance. *Cell Communication and Signaling: CCS*. 2020;**18**(1):59. DOI: 10.1186/s12964-020-0530-4
- [2] Arneth B. Tumor microenvironment. *Medicina (Kaunas, Lithuania)*. 2019;**56**(1):15. DOI: 10.3390/medicina56010015
- [3] Hanahan D, Coussens LM. Accessories to the crime: Functions of cells recruited to the tumor microenvironment. *Cancer Cell*. 2012;**21**(3):309-322. DOI: 10.1016/j.ccr.2012.02.022
- [4] Dudley AC. Tumor endothelial cells. *Cold Spring Harbor Perspectives in Medicine*. 2012;**2**(3):a006536. DOI: 10.1101/cshperspect.a006536
- [5] Salazar N, Zabel BA. Support of tumor endothelial cells by chemokine receptors. *Frontiers in Immunology*. 2019;**10**:147. DOI: 10.3389/fimmu.2019.00147
- [6] Ronca R, Benkheil M, Mitola S, Struyf S, Liekens S. Tumor angiogenesis revisited: Regulators and clinical implications. *Medicinal Research Reviews*. 2017;**37**(6):1231-1274. DOI: 10.1002/med.21452
- [7] Burri PH, Hlushchuk R, Djonov V. Intussusceptive angiogenesis: Its emergence, its characteristics, and its significance. *Developmental Dynamics*. 2004;**231**(3):474-488. DOI: 10.1002/dvdy.20184
- [8] Wolf MJ, Hoos A, Bauer J, Boettcher S, Knust M, Weber A, et al. Endothelial CCR2 signaling induced by colon carcinoma cells enables extravasation via the JAK2-Stat5 and p38MAPK pathway. *Cancer Cell*. 2012;**22**(1):91-105. DOI: 10.1016/j.ccr.2012.05.023
- [9] Birbrair A. Pericyte biology: Development, homeostasis, and disease. *Advances in Experimental Medicine and Biology*. 2018;**1109**:1-3. DOI: 10.1007/978-3-030-02601-1_1
- [10] Aird WC. Endothelial cell heterogeneity. *Cold Spring Harbor Perspectives in Medicine*. 2012;**2**(1):a006429. DOI: 10.1101/cshperspect.a006429
- [11] Gu X, Wei S, Lv X. Circulating tumor cells: From new biological insights to clinical practice. *Signal Transduction and Targeted Therapy*. 2024;**9**:226. DOI: 10.1038/s41392-024-01938-6
- [12] Lian S, Xie R, Ye Y, Lu Y, Cheng Y, Xie X, et al. Dual blockage of both PD-L1 and CD47 enhances immunotherapy against circulating tumor cells. *Scientific Reports*. 2019;**9**(1):4532. DOI: 10.1038/s41598-019-40241-1
- [13] Dong H, Han L, Wu Z-S, Zhang T, Xie J, Ma J, et al. Biostable aptamer rings conjugated for targeting two biomarkers on circulating tumor cells in vivo with great precision. *Chemistry of Materials*. 2017;**29**:10312-10325
- [14] Vidlarova M, Rehulkova A, Stejskal P, Prokopova A, Slavik H, Hajduch M, et al. Recent advances in methods for circulating tumor cell detection. *International Journal of Molecular Sciences*. 2023;**24**(4):3902. DOI: 10.3390/ijms24043902
- [15] Walker C, Mojares E, Del Río HA. Role of extracellular matrix in

development and cancer progression. *International Journal of Molecular Sciences*. 2018;**19**(10):3028. DOI: 10.3390/ijms19103028

[16] Yuan Z, Li Y, Zhang S, Wang X, Dou H, Yu X, et al. Extracellular matrix remodeling in tumor progression and immune escape: From mechanisms to treatments. *Molecular Cancer*. 2023;**22**(1):48. DOI: 10.1186/s12943-023-01744-8

[17] Chakraborty A, Edkins AL. HSP90 as a regulator of extracellular matrix dynamics. *Biochemical Society Transactions*. 2021;**49**(6):2611-2625. DOI: 10.1042/BST20210374

[18] Jahanban-Esfahlan R, Seidi K, Manjili MH, Jahanban-Esfahlan A, Javaheri T, Zare P. Tumor cell dormancy: Threat or opportunity in the fight against cancer. *Cancers (Basel)*. 2019;**11**(8):1207. DOI: 10.3390/cancers11081207

[19] Naba A, Clauser KR, Hoersch S, Liu H, Carr SA, Hynes RO. The matrisome: in silico definition and in vivo characterization by proteomics of normal and tumor extracellular matrices. *Molecular & Cellular Proteomics*. 2012;**11**(4):M111.014647. DOI: 10.1074/mcp.M111.014647. Epub 2011 Dec 9

[20] Naba A, Clauser KR, Ding H, Whittaker CA, Carr SA, Hynes RO. The extracellular matrix: Tools and insights for the “omics” era. *Matrix Biology*. 2016;**49**:10-24. DOI: 10.1016/j.matbio.2015.06.003. Epub 2015 Jul 8

[21] Hong J, Jin HJ, Choi MR, Lim DW, Park J, Kim Y, et al. Matrisomics: Beyond the extracellular matrix for unveiling tumor microenvironment. *Biochimica Et Biophysica Acta. Reviews on Cancer*. 2024;**1879**:189178

[22] Yang C, Liu Z, Zeng X, Wu Q, Liao X, Wang X, et al. Evaluation of the

diagnostic ability of laminin gene family for pancreatic ductal adenocarcinoma. *Aging (Albany NY)*. 2019;**11**(11):3679-3703. DOI: 10.18632/aging.102007

[23] Nieuwenhuis TO, Rosenberg AZ, McCall MN, et al. Tissue, age, sex, and disease patterns of matrisome expression in GTEx transcriptome data. *Scientific Reports*. 2021;**11**:21549. DOI: 10.1038/s41598-021-00943-x

[24] Wang F, Sun W, Zhang J, Fan Y. Cancer associated fibroblast regulation of tumor neoangiogenesis as a therapeutic target in cancer (review). *Oncology Letters*. 2019;**17**:3055-3065. DOI: 10.3892/ol.2019.9973

[25] Bhagwat N, Dulmage K, Pletcher CH, et al. An integrated flow cytometry-based platform for isolation and molecular characterization of circulating tumor single cells and clusters. *Scientific Reports*. 2018;**8**:5035. DOI: 10.1038/s41598-018-23217-5

[26] Caruso S, Poon IKH. Apoptotic cell-derived extracellular vesicles: More than just debris. *Frontiers in Immunology*. 2018;**9**:1486. DOI: 10.3389/fimmu.2018.01486

[27] Sullivan R, Maresh G, Zhang X, Salomon C, Hooper J, Margolin D, et al. The emerging roles of extracellular vesicles as communication vehicles within the tumor microenvironment and beyond. *Frontiers in Endocrinology (Lausanne)*. 2017;**8**:194. DOI: 10.3389/fendo.2017.00194

[28] Kumar MA, Baba SK, Sadida HQ, Marzooqi SA, Jerobin J, Altemani FH, et al. Extracellular vesicles as tools and targets in therapy for diseases. *Signal Transduction and Targeted Therapy*. 2024;**9**(1):27. DOI: 10.1038/s41392-024-01735-1

[29] Joyce JA. Therapeutic targeting of the tumor microenvironment. *Cancer*

Cell. 2005;7(6):513-520. DOI: 10.1016/j.ccr.2005.05.024

[30] Pernot S, Evrard S, Khatib AM. The give-and-take interaction between the tumor microenvironment and immune cells regulating tumor progression and repression. *Frontiers in Immunology*. 2022;13:850856. DOI: 10.3389/fimmu.2022.850856

[31] Ungefroren H, Sebels S, Seidl D, Lehnert H, Hass R. Interaction of tumor cells with the microenvironment. *Cell Communication and Signaling: CCS*. 2011;9:18. DOI: 10.1186/1478-811X-9-18

[32] Bilotta MT, Antignani A, Fitzgerald DJ. Managing the TME to improve the efficacy of cancer therapy. *Frontiers in Immunology*. 2022;13:954992. DOI: 10.3389/fimmu.2022.954992

[33] Knudson KM, Hicks KC, Ozawa Y, et al. Functional and mechanistic advantage of the use of a bifunctional anti-PD-L1/IL-15 superagonist. *Journal for Immunotherapy of Cancer*. 2020;8:e000493. DOI: 10.1136/jitc-2019-000493

[34] Raavé R, van Kuppevelt TH, Daamen WF. Chemotherapeutic drug delivery by tumoral extracellular matrix targeting. *Journal of Controlled Release*. 2018;274:1-8. DOI: 10.1016/j.jconrel.2018.01.029. Epub 2018 Jan 31

[35] Orend G, Chiquet-Ehrismann R. Tenascin-C induced signaling in cancer. *Cancer Letters*. 2006;244(2):143-163. DOI: 10.1016/j.canlet.2006.02.017. Epub 2006 Apr 24

[36] Lin J, Yu Y, Shigdar S, et al. Enhanced antitumor efficacy and reduced systemic toxicity of sulfatide-containing nanoliposomal doxorubicin in a xenograft model of colorectal

cancer. *PLoS One*. 2012;7(11):e49277. DOI: 10.1371/journal.pone.0049277. Epub 2012 Nov 7

[37] Lin J, Shigdar S, Fang DZ, Xiang D, Wei MQ, Danks A, et al. Improved efficacy and reduced toxicity of doxorubicin encapsulated in sulfatide-containing nanoliposome in a glioma model. *PLoS One*. 2014;9(7):e103736. DOI: 10.1371/journal.pone.0103736

[38] Liang H, Li X, Wang B, et al. A collagen-binding EGFR antibody fragment targeting tumors with a collagen-rich extracellular matrix. *Scientific Reports*. 2016;6:18205. DOI: 10.1038/srep18205

[39] Zhang L, Wang Z, Liu K, et al. Targets of tumor microenvironment for potential drug development. *MedComm – Oncology*. 2024;3:e68. DOI: 10.1002/mog2.68

[40] Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. *Nature Reviews. Cancer*. 2020;20(3):174-186. DOI: 10.1038/s41568-019-0238-1

[41] Shao S, Bai W, Zhou P, Luo M, Zhao X, Lei J. Metformin suppresses HIF-1 α expression in cancer-associated fibroblasts to prevent tumor-stromal cross talk in breast cancer. *The FASEB Journal*. 2020;34(8):10860-10870. DOI: 10.1096/fj.202000951RR. Epub 2020 Jun 27

[42] Mehnert JM et al. First-in-human phase 1 study of the arginase inhibitor INCB001158 alone or in combination with pembrolizumab in patients with advanced/metastatic solid tumors. *BMJ Oncology*. 2024;3(1):e000249

[43] Zhang Y, Zhang Z. The history and advances in cancer immunotherapy:

- Understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cellular & Molecular Immunology*. 2020;**17**(8):807-821. DOI: 10.1038/s41423-020-0488-6. Epub 2020 Jul 1
- [44] Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975;**256**:495-497
- [45] Delgado M, Garcia-Sanz JA. Therapeutic monoclonal antibodies against cancer: Present and future. *Cells*. 2023;**12**(24):2837. DOI: 10.3390/cells12242837
- [46] Kothari M, Wanjari A, Acharya S, Karwa V, Chavhan R, Kumar S, et al. A comprehensive review of monoclonal antibodies in modern medicine: Tracing the evolution of a revolutionary therapeutic approach. *Cureus*. 2024;**16**(6):e61983. DOI: 10.7759/cureus.61983
- [47] Hu A, Sun L, Lin H, et al. Harnessing innate immune pathways for therapeutic advancement in cancer. *Signal Transduction and Targeted Therapy*. 2024;**9**(1):68. DOI: 10.1038/s41392-024-01765-9
- [48] Hu S, Liu X, Li T, Li Z, Hu F. LAG3 (CD223) and autoimmunity: Emerging evidence. *Journal of Autoimmunity*. 2020;**112**:102504. DOI: 10.1016/j.jaut.2020.102504. Epub 2020 Jun 20
- [49] Lipson EJ et al. Relatlimab (RELA) plus nivolumab (NIVO) versus NIVO in first-line advanced melanoma: Primary phase III results from RELATIVITY-047 (CA224-047). *JCO*. 2021;**39**:9503-9503. DOI: 10.1200/JCO.2021.39.15_suppl.9503
- [50] Bai R, Cui J. Development of immunotherapy strategies targeting tumor microenvironment is fiercely ongoing. *Frontiers in Immunology*. 2022;**13**:890166. DOI: 10.3389/fimmu.2022.890166. Erratum in: *Front Immunol*. 2022 Aug 11;**13**:1004587. doi: 10.3389/fimmu.2022.1004587
- [51] Philip M, Schietinger A. CD8(+) T cell differentiation and dysfunction in cancer. *Nature Reviews. Immunology*. 2022;**22**(3):209-223
- [52] Ma X et al. Cholesterol induces CD8(+) T cell exhaustion in the tumor microenvironment. *Cell Metabolism*. 2019;**30**(1):143-156.e145
- [53] Muranski P et al. Tumor-specific Th17-polarized cells eradicate large established melanoma. *Blood*. 2008;**112**(1):362-373
- [54] Garon EB et al. A phase Ib open-label, multicenter study of inhaled DV281, a TLR9 agonist, in combination with nivolumab in patients with advanced or metastatic non-small cell lung cancer. *Clinical Cancer Research*. 2021;**27**(17):4566-4573
- [55] Cohen EEW et al. Intralesional SD-101 in combination with pembrolizumab in anti-PD-1 treatment-naïve head and neck squamous cell carcinoma: Results from a multicenter, phase II trial. *Clinical Cancer Research*. 2022;**28**(5):1157-1166
- [56] Khosravi GR, Mostafavi S, Bastan S, Ebrahimi N, Gharibvand RS, Eskandari N. Immunologic tumor microenvironment modulators for turning cold tumors hot. *Cancer Communication (Lond)*. 2024;**44**(5):521-553. DOI: 10.1002/cac2.12539. Epub 2024 Mar 29
- [57] Pauken KE, Dougan M, Rose NR, Lichtman AH, Sharpe AH. Adverse events following cancer immunotherapy: Obstacles and opportunities. *Trends in Immunology*. 2019;**40**(6):511-523

[58] Haanen J, Carbone F, Robert C, Kerr K, Peters S, Larkin J, et al. Corrections to “management of toxicities from immunotherapy: ESMO clinical practice guidelines for diagnosis, treatment and follow-up”. *Annals of Oncology*. 2018;**29**:iv264-iviv6

Immune Cells and Their Role in Immunosuppressive Tumor Microenvironment of Head and Neck Cancer

Eleni Litsou

Abstract

The interaction between immune cells and surrounding tissues that creates the tumor microenvironment may explain the high rates of recurrence and metastatic head and neck squamous cell carcinoma. Notably, head and neck squamous cell carcinoma can evade detection by the immune system while also exhibiting immunomodulatory effects. A variety of cell types comprise the tumor microenvironment, including T cells, cancer-associated stromal fibroblasts, macrophages, B cells, neutrophils, myeloid-derived suppressor cells, mast cells, and natural killer cells. These various cells not only inhabit the tumor but also engage with tumor cells and one another through a complex network. Both adaptive and innate immune cells are crucial for sustaining immune vigilance and regulating tumor growth. In this chapter, we explore the different subsets of immune cells and their roles in fostering an immunosuppressive tumor microenvironment in head and neck squamous cell carcinomas, aiming to enhance our understanding of the numerous factors that significantly influence head and neck squamous cell carcinoma tumorigenesis and progression.

Keywords: head and neck cancers, tumor microenvironment, immunomodulatory cell types, immune escape and evasion, immunecheckpoint inhibitors, immunosuppression, immunotherapy for head and neck cancers

1. Introduction

In recent times, our perspective on cancer has undergone a remarkable transformation. No longer a tumor is considered as a simple mass of malignant cancer cells, but rather as a multifaceted tumor microenvironment (TME), an intricate ecosystem where various subpopulations of cells, ensnared and influenced by cancer cells, come together to create a self-sustaining biological entity. Within this dynamic TME, the stromal component is a veritable tapestry of diverse cell types. We are talking about the likes of cancer-associated fibroblasts (CAFs), neutrophils, tumor-associated macrophages (TAMs), regulatory T cells (Tregs), myeloid-derived suppressor cells

(MDSCs), natural killer cells (NK), platelets, and mast cells, all of which play pivotal roles. These distinct cell populations do not just coexist; they engage in a sophisticated dance of interactions, bursting with communication through a cascade of secreted cytokines, chemokines, growth factors, and the proteins of the extracellular matrix (ECM).

In this chapter, we will delve deeply into these key cell subpopulations and explore the myriad factors that shape the TME. We will also scrutinize their contributions to cancer development, with a keen focus on head and neck squamous cell carcinoma (HNSCC). By examining the variations in stromal composition within the HNSCC microenvironment, we aim to shed light on how these differences influence cancer development and progression. Such insights could unravel the complex mechanisms that dictate the varied responses to therapy, paving the way for identifying potential targets for clinical intervention.

1.1 Head and neck squamous cell carcinoma (HNSCC)

Globally, head and neck cancer (HNC) accounts for 650,000 new cases of cancer and 330,000 deaths annually on average [1]. In that group of diseases, squamous cell carcinoma constitutes the majority of head and neck carcinomas. Independently of the exact site, HNSCC has multiple similarities regarding their epidemiologic, demographic, pathologic, and sometimes, clinical features [2].

HNSCC is composed of a group of malignant neoplasms that display a great degree of heterogeneity. They are thought to arise from the non-keratinizing epithelium of the upper aerodigestive tract. Potential sites of HNSCCs include the oral cavity, oropharynx, hypopharynx, larynx, paranasal sinuses, and nasal cavity, but also the cervical esophagus, thyroid glands, and salivary glands. Most head and neck malignancies, though, originate from the mucosal epithelium in the oral cavity [3].

The head and neck region, particularly the salivary glands, harbors a wide variety of carcinomas, including those arising from both major and minor salivary glands. These malignancies can originate from different cellular components within the salivary glands, with distinct histological and molecular characteristics [4].

1.2 Epidemiological data and environmental risk factors

Similar to other types of cancer, diagnosis of HNSCCs often happens too late. This happens due to the absence of precancerous lesions or other early stage, easily identifiable symptoms [5]. Early-stage HNSCCs might present vague symptoms such as a sore throat, difficulty swallowing, or a persistent lump, which can be mistaken for less serious conditions, delaying diagnosis and treatment [6].

The majority of HNSCCs are associated with human behaviors. Alcohol and tobacco consumption is thought to be responsible for approximately 75% of cases. At the same time, there seems to be a strong link between pharyngeal cancer and human papillomavirus (HPV) infection, mainly high-risk variants such as HPV-16, 18, and 33 [2].

There are additional specifics of risk factors that may increase or decrease the risk for HNSCCs, such as the fact that smoking black tobacco poses a higher risk than smoking blond tobacco; the same is true for smoking at a young age, smoking a high number of cigarettes a day, and also deep smoke inhalation [7].

During the past decades, new epidemiological trends have been observed regarding HNSCCs. There has been a rise in HPV-associated oropharyngeal cancers,

particularly among younger, non-smoking individuals, while the incidence of tobacco-related cancers has decreased. Additionally, there has been a shift in the predominant location of HNSCCs from the larynx to the oropharynx, and survival rates have improved due to advances in early detection and treatment [8]. These subsite-specific epidemiological trends have been attributed to shifts in societal factors that have resulted in changes in exposure to two divergent but complementary classes of HNSCC risk factors: (1) tobacco and alcohol consumption and (2) HPV infection [9].

The exact reasons and causes for the societal trends that drive these epidemiological trends cannot be accurately pinpointed. However, it is thought that various public health campaigns are largely credited with driving decreases in tobacco and alcohol consumption [10]. At the same time, sexual practices that may introduce the risk of contracting sexually transmitted pathogens, like HPV, have been linked to the rise in HPV-associated cancers, including oropharyngeal squamous cell carcinoma (OPSCC) [11].

Since HPV is, in most cases, cleared within 18 months, it is hypothesized that persistent infection would be necessary for HPV-associated malignancies to form [12]. As mentioned earlier, OPSCC seems to be linked to HPV infection. Interestingly, HPV-positive OPSCC is considered to be a separate neoplastic entity, with its own individual characteristics not just on a molecular and epidemiological but also on a clinical level [13]. HPV presents a major risk to public health, and so the development of vaccines for various strains of the virus has been in the works for multiple decades. The Food and Drug Administration (FDA) approved vaccines in 2006 and 2014 that target the very common HPV strains 16/18. These vaccines are thought to be able to prevent cases of HPV-positive HNSCCs [2].

There are distinct differences between HPV-positive and HPV-negative HNSCCs. When it comes to prognosis, HPV-positive HNSCC is generally more favorable. This is attributed to the unique biological characteristics that HPV-positive HNSCC tumor cells hold. They are considered to be more susceptible to being suppressed by the immune response as well as more sensitive to treatment methods such as radiotherapy and chemotherapy [14]. HPV-negative HNSCC lacks the immunogenicity associated with HPV, making it less responsive to radiotherapy and chemotherapy. Its biological behavior is more aggressive, with a higher tendency for local infiltration and distant metastasis.

At present, the targeted therapy drug cetuximab and immunotherapy PD-1/PD-L1 (programmed death-ligand 1/programmed cell death protein 1) inhibitors (pembrolizumab, nivolumab) have been approved for the treatment of recurrent or metastatic (R/M) HNSCC [15]. Inhibitors of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) can enhance the immune response of T cells and have already been tested in combination with PD-1 and PD-L1 inhibitors in clinical trials [16]. Immunotherapy for HNSCC seems to be a promising prospect and will be discussed in more detail in later chapters.

2. Tumor microenvironment

The TME is a dynamic and complex system consisting of cancer cells, stromal tissue, immune cells, blood vessels, fibroblasts, and signaling molecules, all embedded in the ECM [17]. These components engage in a continuous exchange of signals that support tumor growth, survival, and invasion into healthy tissues. The TME plays a critical role in cancer development, influencing not only how tumors grow but also how they resist treatments, leading to poor outcomes for many patients [16].

A key feature of the TME is its hypoxic nature. As tumors grow beyond the capacity of existing blood vessels to supply oxygen and nutrients, regions within the tumor become deprived of oxygen, leading to hypoxia. In response, the tumor promotes angiogenesis, the formation of new blood vessels, to meet its demands [18]. Hypoxia also triggers the upregulation of hypoxia-inducible factors (HIFs), which alter cellular metabolism, pushing cancer cells to favor glycolysis over aerobic respiration. This metabolic shift, known as the Warburg effect, creates an acidic microenvironment that promotes cancer cell survival and aggressiveness [19].

The altered vasculature in the TME is often disorganized, with leaky and poorly structured blood vessels. These abnormal blood vessels contribute to an inefficient supply of oxygen and nutrients, further exacerbating hypoxia [20]. Additionally, the leaky vasculature allows macromolecules, including therapeutic agents, to accumulate in the tumor tissue, a phenomenon known as the enhanced permeability and retention effect. While this offers an opportunity for targeted drug delivery, the irregular structure of the vasculature can also limit the effectiveness of treatments by impairing drug penetration and distribution within the tumor [21].

The TME represents a highly dynamic and interactive ecosystem, wherein cancer cells engage in continuous bidirectional communication with a variety of surrounding stromal components. This intricate crosstalk enables malignant cells to reprogram nearby non-neoplastic elements, inducing the secretion of paracrine signals that support tumor cell proliferation, survival, and dissemination. The TME comprises a diverse range of nonmalignant cell types, including immune cells, fibroblasts, mesenchymal stem cells, endothelial cells, peripheral nerve fibers, and cells derived from the bone marrow and hematopoietic lineages (**Figure 1**) [22, 23]. Strikingly, these stromal and immune constituents can account for up to 90% of the total tumor mass, underscoring their significant role in the tumor architecture.

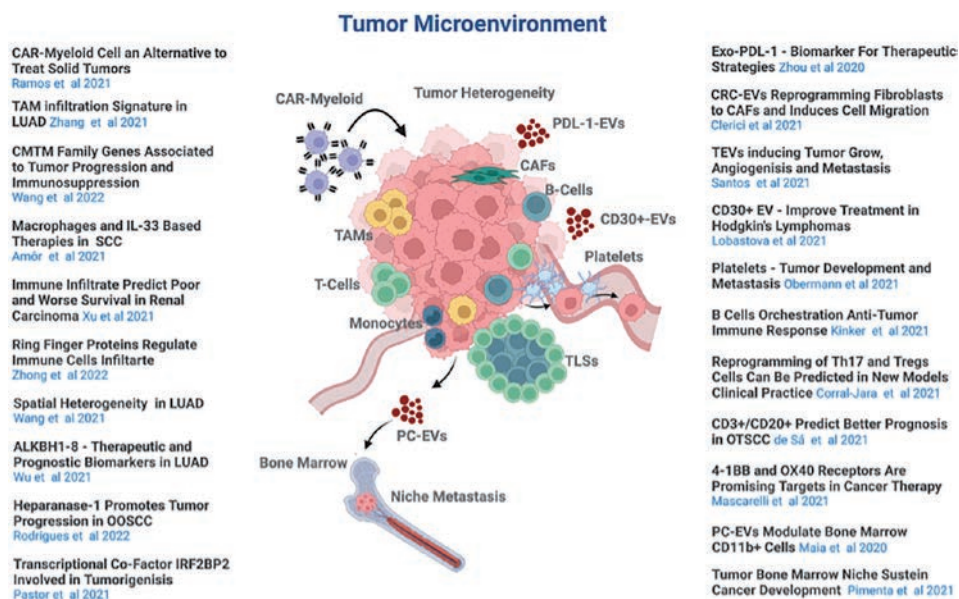


Figure 1. The tumor microenvironment. A schematic view of the tumor microenvironment components and all scientific contributions in this topic research. The picture was created with BioRender.com [23].

Although once considered passive bystanders—or even physical barriers to tumor growth—emerging evidence increasingly highlights that these nonmalignant stromal components are active participants in tumorigenesis, metastatic dissemination, and resistance to therapy [19]. A particularly critical aspect of the TME is its immunosuppressive nature, which impairs the host's antitumor immune response. TAMs, Tregs, and other immune populations are frequently hijacked by the tumor to adopt protumorigenic phenotypes, thereby fostering immune evasion and contributing to a microenvironment that shields the tumor from immune-mediated destruction [24].

This immune tolerance, compounded by the tumor's ability to exploit stromal elements for metabolic and structural support, complicates therapeutic interventions and facilitates cancer persistence. Consequently, deciphering the complex cellular and molecular interplay within the TME is paramount for the development of innovative therapeutic strategies aimed not only at targeting malignant cells but also at disrupting the supportive niche that sustains them [24].

3. Immune cells and their role in regulating the tumor microenvironment

The high potential for metastasis and recurrence in HNSCC is largely driven by the complex interactions between the TME and nearby immune cells. Normally, the immune system can detect and destroy cancer cells, but tumor cells often develop ways to evade this detection, creating an environment that promotes their growth and survival [6]. HNSCC not only escapes immune detection but also suppresses immune responses.

One key method HNSCC uses to evade the immune system is by reducing human leukocyte antigen (HLA) expression, which impairs T cells' ability to recognize and attack cancer cells. Additionally, the TME in HNSCC hinders the function of tumor-infiltrating lymphocytes (TILs), weakening the immune system's ability to fight the tumor [24].

As was mentioned before, the TME is a complex and diverse ecosystem made up of various immune and stromal cells, such as CAFs, T cells, B cells, neutrophils, macrophages, MDSCs, NK cells, and mast cells (**Figure 2**). These cells form networks that influence both tumor growth and immune modulation. While immune cells like NK and CD8⁺ T cells play important roles in tumor suppression, others, such as MDSCs and macrophages, though counterintuitive, can actually support tumor growth [24].

By understanding the complex interactions within the TME, new therapies can be developed to enhance immune responses and counteract the factors that allow HNSCC to progress.

3.1 T cells (CD4⁺, CD8⁺, Treg)

T cells, crucial components of the adaptive immune system, are classified into CD4⁺ and CD8⁺ T cells based on the cluster of differentiation (CD). CD8⁺ TILs play a key role in antitumor immunity by directly killing tumor cells through the release of cytolytic molecules like perforin and granzymes. Both activated CD4⁺ and CD8⁺ TILs also contribute to tumor suppression by secreting cytokines such as interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α), which not only have direct tumor-killing effects but also recruit and activate other immune cells to enhance cytotoxic responses [25, 26]. CD8⁺ T cells primarily recognize tumor antigens presented by major histocompatibility complex (MHC) class I molecules [27].

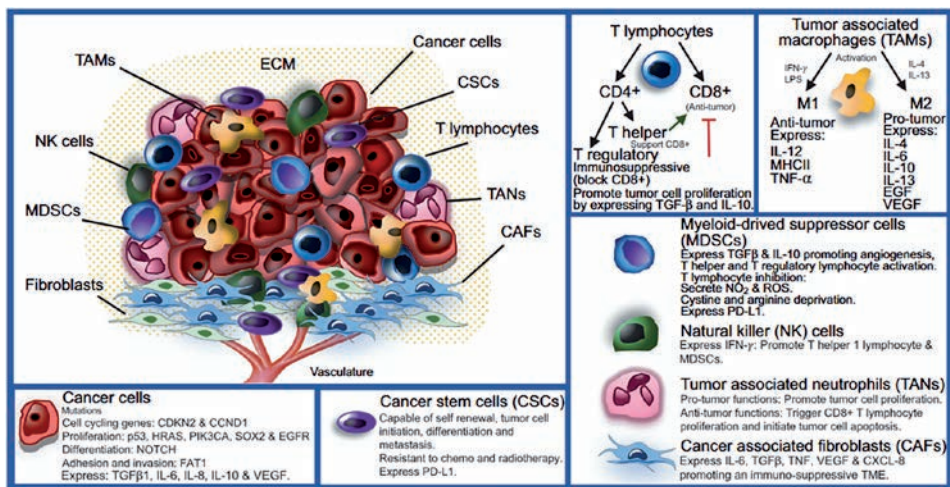


Figure 2.

The TME of HNSCC. The TME is composed of cancer cells and heterogeneous nonmalignant cells integrated in a complex extracellular matrix (ECM). The main cellular components of the TME are T lymphocytes, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), natural killer (NK) cells, tumor-associated neutrophils (TANs), and cancer-associated fibroblasts (CAFs). Immune cells play a key role in tumor cell growth and dissemination [24].

After receiving costimulatory signals and cytokine stimulation, particularly from interleukin-2 (IL-2), CD8⁺ T cells differentiate into cytotoxic T lymphocytes (CTLs) capable of exerting potent cytotoxic effects and secreting IFN- γ [28]. IFN- γ enhances antitumor immunity by boosting APCs' activity, promoting macrophage phagocytosis, and modulating other immune cells, emphasizing its vital role in both antiviral and antitumor responses [29].

In a study analyzing oral squamous cell carcinoma (OSCC) patient samples, it was found that the majority of intratumoral TILs were CD8⁺ T cells, while 67% of patients had intratumoral CD4⁺ TILs [30]. A higher frequency of peritumoral CD8⁺ TILs was associated with better clinical outcomes, including smaller tumor size and a lower probability of lymph node metastasis in HNSCC patients; however, this did not correlate with patient survival. The CD4:CD8 TIL ratio was reported as 0.745 and was found to be higher in larger and more advanced-stage tumors. Despite the association between peritumoral CD8⁺ TILs and clinical parameters, neither the frequency of intratumoral CD8⁺ TILs nor the CD4:CD8 ratio was linked to clinical outcomes in OSCC [30].

A systematic review and meta-analysis further confirmed that CD3⁺ and CD8⁺ TILs have a favorable prognostic role in HNSCC outcomes and found that Tregs that express the transcription factor forkhead box protein 3 (FoxP3⁺ Tregs) also correlated with improved overall survival (OS). The review showed that high infiltration of CD3⁺ TILs was linked to improved OS and disease-free survival (DFS), while elevated CD8⁺ TIL levels were associated with better OS, DFS, and locoregional control (LRC). Increased CD4⁺ TIL infiltration was also tied to favorable OS and LRC. Interestingly, higher counts of FoxP3⁺ Tregs, typically considered Tregs, were unexpectedly associated with improved OS, highlighting the complex role these cells play in tumor immunity [31].

These findings suggest that while peritumoral CD8⁺ TILs may influence tumor characteristics, their presence does not necessarily predict survival, and the CD4:CD8

ratio may be more indicative of tumor progression rather than prognosis. The potential of TILs, especially CD3+ and CD8+ subsets, as favorable prognostic biomarkers in HNSCC is evident. The study also emphasizes the importance of standardized evaluation methods and calls for further research to fully understand the role of FoxP3+ Tregs in cancer prognosis.

3.2 Tumor-associated macrophages (TAMs)

TAMs are essential immune components of the TME, originating from bone marrow-derived monocytes [32, 33]. Far from being passive, they actively contribute to cancer-related inflammation as infiltrating inflammatory cells [34]. TAMs facilitate tumor progression by degrading the ECM, remodeling the TME, enhancing cancer cell motility, and promoting angiogenesis [34]. Their recruitment is mediated by cytokines and growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), interleukin-10 (IL-10), granulocyte macrophage colony-stimulating factor (GM-CSF), and various chemokines [32].

These macrophages secrete protumorigenic factors, notably VEGF80 and EGF81, which drive neovascularization, tumor invasion, and metastasis [35, 36]. Functionally, TAMs can polarize into two phenotypes: M1 and M2. M1 macrophages, activated by IFN- γ and microbial stimuli, exhibit proinflammatory and antitumor properties, characterized by high levels of interleukin-12 (IL-12), MHC class II, and TNF- α . Conversely, M2 macrophages, stimulated by interleukin-4 (IL-4) and interleukin-13 (IL-13), are immunosuppressive, producing high IL-10 and expressing low levels of IL-12 and MHC-II [24, 37]. This phenotypic plasticity is central to their dual role in immune regulation and tumor progression.

The expression of PD-L1 on these macrophages plays a pivotal role in sustaining an immunosuppressive environment by delivering continuous inhibitory signals that foster an immunosuppressive phenotype [38].

A study focusing on oral tongue squamous cell carcinoma (OTSCC) found that activated CD4+ T helper cells enhanced PD-L1 expression on macrophages via IFN- γ signaling [39]. This upregulation of PD-L1 may act as a protective mechanism for macrophages and dendritic cells (DCs), shielding them from apoptosis [40]. Furthermore, OSCC tumor cells were observed to induce PD-L1 expression in TAMs through IL-10, which in turn induced T-cell apoptosis and was associated with a poorer prognosis for patients [41, 42].

In HPV-positive HNSCC, TAMs play a significant role in adaptive resistance by suppressing tumor-specific T-cell responses through the expression of PD-L1 at the boundary between tumor nests and the adjacent inflammatory stroma [43]. In these HPV-positive HNSCC cases, macrophages can directly induce PD-L1 expression on tumor cells, reinforcing the immunosuppressive environment and potentially aiding the tumor in evading immune detection [44].

TAMs are notably concentrated in hypoxic regions of HNSCC, areas typically linked to more aggressive tumor behavior. Elevated TAM infiltration in these zones correlates strongly with poor OS [45]. This is largely due to their polarization toward the immunosuppressive M2 phenotype, which suppresses antitumor immunity through the release of anti-inflammatory cytokines and inhibition of T-cell responses [46]. Given their pivotal role in promoting tumor growth and immune evasion, TAMs have emerged as promising therapeutic targets. Current strategies focus on either depleting TAMs or reprogramming them toward a proinflammatory M1 phenotype

to reinstate effective antitumor immunity [38]. These approaches represent a potential breakthrough in improving therapeutic outcomes for HNSCC and other solid malignancies.

3.3 Natural killer (NK) cells

NK cells play a crucial role in both innate and adaptive immune responses, acting as a key bridge between the two systems. Their rapid secretion of IFN- γ facilitates the recruitment and activation of T helper type 1 (Th1) cells and MDSCs, amplifying the antitumor immune response [47]. NK cells identify abnormal cells by detecting imbalances between activating and inhibitory signals, primarily through interactions with MHC class I molecules [48]. Tumor cells often escape immune detection by downregulating MHC-I or upregulating stress-induced ligands like NKG2D ligands, increasing their susceptibility to NK-mediated cytotoxicity. Through the release of perforin and granzyme B, NK cells directly induce apoptosis in target cells. Additionally, they secrete immunostimulatory cytokines such as IL-2, IL-12, IL-15, IFN- γ , and TNF- α , which further promote adaptive immunity [47].

Transcriptomic analyses have revealed that HNSCC, particularly HPV-positive (HPV+) tumors, ranks among the most immune-infiltrated cancers, with the highest median levels of cytotoxic CD56 dim NK cells (express CD56 at low levels) across tumor types [49]. Elevated CD56 dim NK cell infiltration is associated with improved disease-free and OS, independent of HPV status. Moreover, peripheral NK activity correlates with reduced metastatic spread in pharyngeal cancers [49, 50].

However, tumor-infiltrating NK cells in HNSCC differ phenotypically from their circulating counterparts. Within the TME, they often express higher levels of the inhibitory receptor NKG2A and lower levels of inhibitory killer-cell immunoglobulin-like receptors (KIRs) such as KIR3DL1 and KIR2DL1/2/3 [50, 51]. While CD56 dim cells dominate, HNSCC tumors are also enriched with immature, cytokine-secreting CD56 bright, CD16 dim NK cells lacking CD57 [52]. Notably, NK cells often accumulate in the stromal regions rather than in close proximity to tumor nests, limiting their direct tumor-killing capacity.

Despite this spatial limitation, their dual capacity for direct cytotoxicity and immune modulation underscores their value as targets for immunotherapy. Variability in antibody-dependent cellular cytotoxicity (ADCC) among patients and increasing interest in NK checkpoint receptors further support the exploration of NK-based therapies in HNSCC. Elevated NK cell presence has consistently been linked to better clinical outcomes, reinforcing their therapeutic potential.

3.4 Myeloid-derived suppressor cells (MDSCs)

MDSCs are a heterogeneous population of immature myeloid cells that arise from hematopoietic stem cells (HSCs) during the process of myelopoiesis. Known for their potent immunosuppressive properties, MDSCs play a critical role in protecting tumor cells from immune attack and undermining antitumor responses. They are typically classified into two main subsets: monocytic MDSCs (M-MDSCs) and polymorphonuclear or granulocytic MDSCs (PMN-MDSCs) [53]. Although PMN-MDSCs phenotypically resemble neutrophils, they lack neutrophils' typical immunostimulatory and antitumor functions.

In HNSCC, elevated MDSC levels in both circulation and tumor tissue have been linked to poor therapeutic response and reduced OS [54, 55]. They are increasingly

recognized as valuable prognostic biomarkers in cancer. Initial research in HNSCC demonstrated that MDSCs suppress activated T cells and produce nitric oxide (NO) and reactive oxygen species (ROS), which nitrate the T-cell receptor (TCR) and disrupt antigen recognition via MHC, thus impairing T-cell activation [56].

MDSCs are actively recruited into the TME via inflammatory cytokines and growth factors such as IL-1 β , IL-6, IL-10, GM-CSF, and VEGF [57, 58]. Within the TME, they contribute to tumor progression by promoting angiogenesis and inducing immunosuppressive cell populations like Tregs and T helper type 2 (Th2) cells. They also interfere with antigen presentation by outcompeting DCs and TAMs, compromising effective T-cell priming.

In addition, MDSCs deplete cysteine, an amino acid essential for T-cell activation, and metabolize arginine via arginase activity, producing immunosuppressive NO and ROS. These metabolites further disrupt T-cell receptor signaling and cytotoxic function [59]. Furthermore, MDSCs express PD-L1, engaging PD-1 on T cells to suppress their activity and promote immune escape [60]. Emerging evidence also suggests that MDSCs impair NK cell activity, thereby reducing the efficacy of NK cell-based therapies in HNSCC [34].

Age-related increases in basal MDSC levels may further explain the correlation between aging and increased tumor incidence and progression [61]. Therapeutically, strategies such as MDSC depletion (e.g., with retinoic acid, gemcitabine), signal transducer and activator of transcription 3 (STAT3) inhibition, or antibody-based targeting have shown promise in restoring immune surveillance and enhancing immunotherapy efficacy [62].

Overall, MDSCs are central to the establishment of an immunosuppressive tumor niche in HNSCC, making them a critical target in efforts to enhance antitumor immunity and improve clinical outcomes.

3.5 Cancer-associated fibroblasts (CAFs)

CAFs are key stromal elements in the TME, especially in HNSCC, where they actively support tumor growth, immune suppression, and resistance to therapy. Although nonmalignant, CAFs resemble myofibroblasts involved in wound healing and fibrosis and originate from diverse sources, including mesenchymal stem cells, fibrocytes, pericytes, and epithelial cells via epithelial-to-mesenchymal transition (EMT) [63]. This diversity has led to the recognition of multiple CAF subtypes with distinct functions.

CAF identification relies on markers such as α -smooth muscle actin (α -SMA), vimentin, fibroblast activation protein (FAP), and fibroblast-specific protein 1 (FSP1), although their expression varies across fibroblast populations [63]. In HNSCC, transforming growth factor- β (TGF- β), often in conjunction with interleukin-1 β (IL-1 β), drives the transition of normal fibroblasts into protumorigenic myofibroblasts [64]. Additionally, tumor cells secrete FAP and PDGF-A, further promoting CAF accumulation and activation within the TME [63].

Functionally, CAFs contribute to angiogenesis, tumor proliferation, invasion, and metastasis by releasing cytokines and growth factors such as interleukin-6 (IL-6), TGF- β 1, TNF, VEGF, and C-X-C motif chemokine ligand 8 (CXCL8). Importantly, CAFs also modulate the immune landscape by upregulating PD-L1, leading to suppression of T-cell activity, increased T regulatory cell (Treg) infiltration, and induction of T-cell apoptosis [65]. PD-L1 expression is observed in around 40% of HNSCC tissues, although its prognostic significance remains unclear [66].

A reciprocal relationship exists between CAFs and tumor cells regarding immune checkpoint regulation. HPV-positive HNSCC cells can increase PD-L1 and PD-L2 expression on fibroblasts via toll-like receptor 9 (TLR9)-mediated signaling, while fibroblasts can in turn elevate PD-L1 levels on tumor cells [44]. TGF- β has also been shown to induce PD-L1 expression on fibroblasts in lung cancer models and suppress T-cell responses through PD-L1-enriched exosomes [67]. PD-L1 overexpression on CAFs has been directly linked to T-cell apoptosis, contributing to an immunosuppressive TME.

While CAFs clearly play a role in immune evasion, it remains unclear whether PD-L1+ CAFs act through direct cell-cell interactions or via secreted factors [68]. Further studies are needed to clarify these mechanisms. Nonetheless, due to their central role in tumor progression and immune regulation, CAFs are increasingly being investigated as promising therapeutic targets in HNSCC. Disrupting CAF function or altering their immunosuppressive phenotype may enhance immune surveillance and improve treatment outcomes.

3.6 Cytokine signaling in HNSCC: Roles in immune modulation and tumor progression

The HNSCC TME is a heterogeneous complex of cellular and noncellular components. In addition to providing intermediate metabolites and nutrients to the tumor cells, the immune stromal cells secrete a diverse array of cytokines, chemokines, and growth factors (**Figure 3**) that support tumor growth, progression, metastasis, and host immunosuppression and promote the development of aggressive tumors [69]. Cytokines play multifaceted roles in cancer biology and have been broadly categorized based on their function in (1) regulating tumor cell proliferation, (2) serving as prognostic markers, and (3) acting as potential immunotherapeutic targets. In HNSCC, cytokine dysregulation contributes significantly to immune evasion and tumor progression.

TGF- β , a cytokine abundantly expressed in HNSCC, plays a pivotal role in promoting Treg differentiation and suppressing NK cell activity, contributing to immunosuppression within the TME [70]. Alongside TGF- β , IL-6 and IL-10 have been identified as major tumor-promoting cytokines [71]. These molecules suppress STAT1 signaling, which is critical for antitumor immunity, and instead activate STAT3, leading to inhibition of T-lymphocyte function, impaired maturation of DCs, suppression of NK cells and neutrophils, and promotion of an immunosuppressive milieu.

IL-10, through STAT3 activation, also downregulates IL-12, a cytokine essential for Th1 differentiation and cytotoxic responses [72]. This promotes the expansion of Treg cells, which are found in elevated levels in HNSCC patients, contributing to systemic immunosuppression. Notably, Treg accumulation inversely correlates with CD8+ cytotoxic T-cell infiltration [73] and is further increased following oncologic treatments, which has been associated with resistance to anti-PD-1 immunotherapies and poorer clinical outcomes [74].

A suppressive subpopulation of CD4+ CD25+ Tregs has been implicated in tumor-supportive autoimmunity [74]. Following IL-12 engagement with CD25 receptors, these cells secrete TGF- β and IL-10, reinforcing tumor immune escape and progression [75]. Conversely, TAMs with an M1 phenotype exhibit antitumor activity via secretion of IL-12, TNF- α , and IFN- γ . However, in the HNSCC microenvironment, TAMs often polarize to the M2 phenotype, secreting IL-4 and IL-13, thus promoting a Th2-skewed immune response and tumor progression [23, 24].

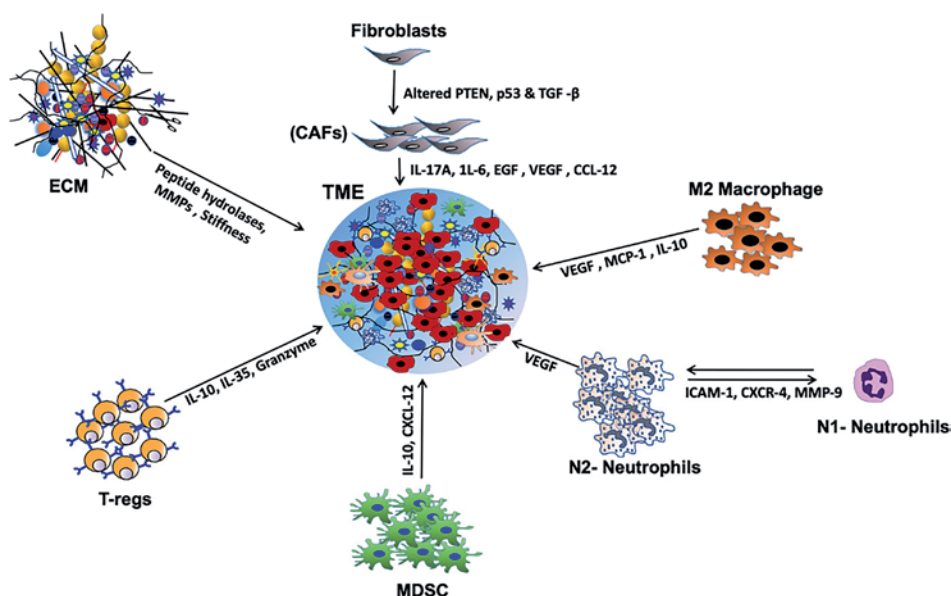


Figure 3. Chemokine and cytokine-mediated crosstalk in head and neck squamous cell carcinoma (HNSCC) tumor microenvironment (TME). Cytokines and chemokines secreted by a variety of stromal cells affect tumor cell growth, proliferation, and metastasis in many ways. By inducing immunosuppressive TME, they promote immune evasion and metastasis. Many chemokines and cytokines help degrade extracellular matrix (ECM) proteins, induce angiogenesis, and thereby promote invasion and metastasis [69].

VEGF, overexpressed in most HNSCC tumors, not only drives angiogenesis but also impairs DC maturation, leading to T-cell dysfunction and immune tolerance. TLRs, when activated, stimulate proinflammatory cytokines such as $\text{TNF-}\alpha$ and $\text{IFN-}\gamma$, contributing to a Th1-dominated antitumor immune response. However, this is often outweighed by Th2 cytokines (IL-4, IL-6, IL-10) in HNSCC, creating an immunosuppressive barrier to effective immunotherapy [75]. Elevated levels of EGF, IL-6, and IL-10 have consistently been associated with poor clinical outcomes [76].

4. Mechanisms of immunosuppression in the tumor microenvironment

The immune system is crucial when it comes to the development, establishment, and progression of HNSCC. HNSCC cells have mastered the art of evading the host immune response, skillfully tweaking their own immunogenic nature, producing immunosuppressive mediators, and promoting immunomodulatory cell types. The tumor manipulates its microenvironment in a way that can twist the immune system into a tool for its own gain, promoting metastasis, sparking angiogenesis, and driving growth.

During tumorigenesis, rapid malignant cell proliferation outpaces vascular development, resulting in regions of hypoxia within the TME. Although hypoxia has traditionally been associated with growth inhibition, emerging evidence indicates it promotes tumor progression and immune escape mechanisms [77]. There is an inverse correlation between hypoxia-related gene expression and antitumoral immune responses in HNC. Hypoxia drives immune suppression via multiple mechanisms. It may be achieved through HIF1 α -mediated activation of inhibitory pathways,

including CD47, the adenosine-generating enzymes CD73 and CD39, and immune checkpoint receptors such as PD-L1. Tumor cell metabolic adaptations to hypoxia, such as increased glucose uptake and lactate production, also act to promote and perpetuate an immunosuppressive microenvironment. The balance of antitumoral versus immunosuppressive function of myeloid cells, including MDSCs and TAMs, may be modulated by tumor hypoxia, which favors suppression. Hypoxia can tip the balance of cytokine/chemokine/effector molecule production toward those with protumoral functions (IL-10, VEGF, arginase, and iNOS) and upregulate expression of T-cell inhibitory ligands (PD-L1).

Key mediators of the hypoxic response are the HIFs, which are upregulated in oxygen-deficient regions. HIFs orchestrate transcriptional programs that regulate metabolism, proliferation, angiogenesis, and tissue remodeling [78]. In HNSCC, especially in HPV-negative cases, hypoxia has been linked to overexpression of EGF receptor (EGFR)—a known activator of HIFs—and both EGFR and HIF overexpression are associated with poor prognosis. Importantly, hypoxia also contributes to immunosuppression [79]. It promotes the secretion of immunomodulatory cytokines, reduces T-lymphocyte proliferation, facilitates the recruitment of suppressive immune cells, such as Tregs and MDSCs, and enhances PD-L1 expression on tumor and stromal cells, collectively impairing antitumor immune responses. These findings underscore the dual role of hypoxia in driving tumor aggressiveness and undermining immunosurveillance, making it a critical therapeutic target in HNSCC [77].

There are various paths through which tumor cells avoid immune detection, including the release of immunosuppressive molecules such as TGF- β , IL-10, and PD-L1 [80]. These molecules inhibit the activity of CTLs and NK cells, promote the growth of Tregs, and suppress overall cell-mediated immunity. Additionally, inhibitory receptors (IRs) are expressed by tumor cells such as CTLA-4 and PD-1 on activated T cells, along with their ligands (**Figure 4**) [81]. As a result of the increased expression of these IRs, T cells are suppressed, proliferation is reduced, and immune surveillance is weakened, allowing tumor cells to evade destruction by the immune system [81].

Since tumor cells evade immune detection, they can then proliferate, invade surrounding tissues, and metastasize more efficiently. Immunoediting is further able to refine the understanding of tumorigenesis and disease progression by highlighting the immune system's dual role. While the immune system can initiate an antitumor response, tumors may adapt and exploit immune mechanisms to enhance their own survival and progression [82].

4.1 Regulatory molecules and signaling pathways PD-1/PD-L1

PD-1 is an immunosuppressive receptor located on the surface of various immune cells, including T cells, B cells, NK cells, and myeloid cells. With a molecular weight of around 50–55 kDa, PD-1 plays a crucial role in modulating T-cell activity within tissues, primarily acting to suppress overactive immune responses. This function helps to prevent autoimmunity but also impairs the ability of T cells to launch effective antitumor responses in malignancies [83, 84]. PD-1 interacts with its ligands, PD-L1 and PD-L2, to mediate these immunosuppressive effects.

PD-L1 is expressed across a wide range of hematopoietic cells, such as T cells, B cells, macrophages, DCs, and neutrophils, as well as non-hematopoietic tissues, including the heart, pancreas, placenta, liver, lung, muscle, and skin. Additionally, it is found on antigen-presenting cells (APCs) as opposed to PD-L2 expression, which is

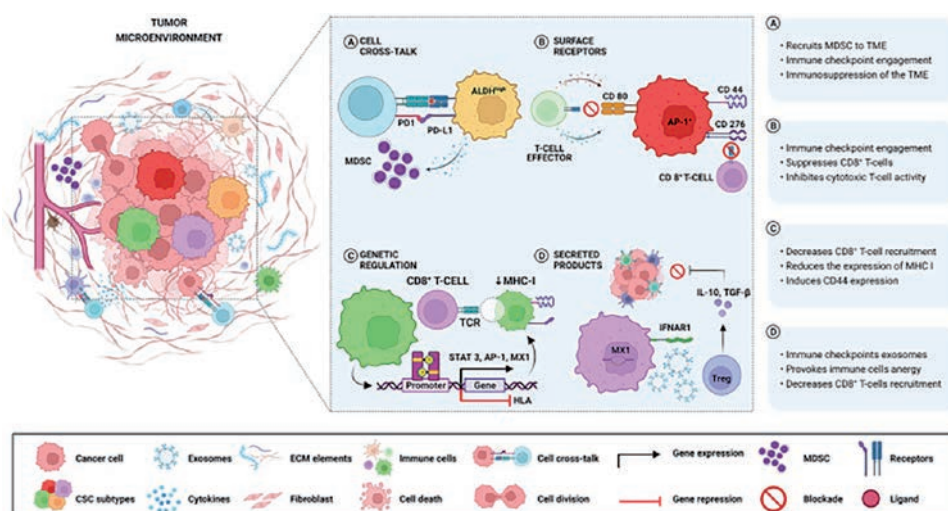


Figure 4. Different mechanisms involved in immune escape are driven by oral and HNSCs. (A) ALDH^{high} CSCs exhibit higher levels of PD-L1 and recruit MDSCs with a suppressive role, causing negative regulation of immune responses in the TME. (B) CSCs expressing CD276 and AP-1 create continuous positive feedback that enables immune evasion by suppressing CD8⁺ T cells. After TGF- β stimulation, CSCs express CD80 and inhibit T-cell cytotoxicity, leading to resistance to T-cell immunotherapy. (C) The activation of the STAT3 pathway is related to the expression of PD-L1 in CD44⁺ cells, resulting in immune escape played by CSCs. Downregulation of HLA genes in CSCs decreases the expression of MHC class I, causing non-recognition by T cells. (D) High expression of MX1 in CSCs decreases CD8⁺ T cells infiltration concomitant with IFNAR1 expression, associated with the release of exosomes containing immune checkpoint receptors. Noteworthy, the same CSCs can activate more than one immune avoidance mechanism [81].

more restricted, primarily appearing on activated macrophages and DCs. When PD-1 binds to PD-L1 or PD-L2, it inhibits T-cell activation by interfering with TCR signaling [84]. Significantly, elevated PD-L1 expression is often observed in cancer cells and the tumor stroma, which aids tumors in evading immune detection [85].

By expanding on the basic knowledge of PD-1 and its ligands, a deeper analysis of their molecular structures and functions can reveal their essential roles in immune regulation [86]. PD-1 and PD-L1 possess structural characteristics that contribute to their ability to mediate immune homeostasis and play a pivotal role in immune evasion by tumors. These insights also highlight their implications in cancer progression and the mechanisms behind immune suppression (**Figure 5**) [87].

PD-L1 expression levels are often found to be elevated in tumor cells. When PD-L1 on tumor cells interacts with PD-1 on immune cells, particularly T cells, it initiates a signaling cascade that begins with the phosphorylation of PD-1's immunoreceptor tyrosine-based inhibitory motifs (ITIMs) and immunoreceptor tyrosine-based switch motifs (ITSMs). This phosphorylation leads to recruitment and activation of the protein tyrosine phosphatase SHP-2, which in turn sends inhibitory signals that dampen T-cell activity [88]. Through these signals, interferon release is inhibited as well as TNF- α and VEGF release, thereby impairing T cells' ability to mount an effective antitumor response. As a result, immune detection and destruction can be evaded by tumor cells [89].

In addition to PD-L1, another ligand for PD-1, PD-L2, also contributes to immune suppression by transmitting inhibitory signals upon binding to PD-1 [90]. However, PD-L2's expression is more limited compared to PD-L1, and its regulatory functions are more intricate [91]. PD-L2 may play a different role from PD-L1 in certain

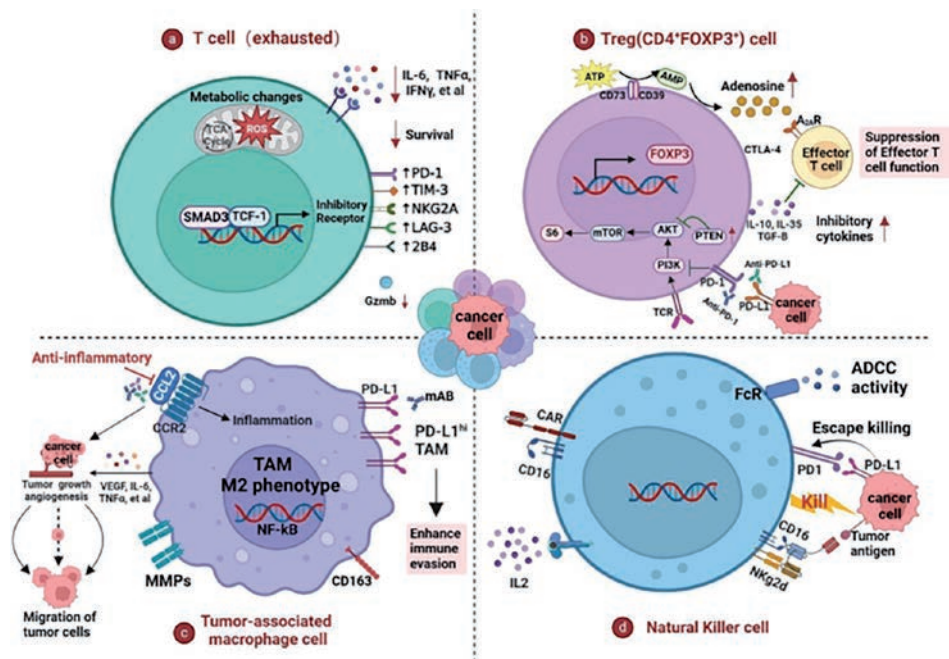


Figure 5. The regulation of PD-1/PD-L1 signaling on immune cells. *a.* The PD-1/PD-L1 pathway promotes the exhaustion and apoptosis of effector T cells. T_{ex} cells are characterized by increased expression of highly inhibitory receptors, including PD-1, LAG3, and TIGIT; decreased cytokine production such as TNF, IL-2, and IFN- γ ; metabolic alterations; and impaired proliferative capacity and survival. *b.* PD-1/PD-L1 promotes the generation and development of induced Tregs (iTregs) by reducing the phosphorylation of PI3K/Akt/mTOR and S6 while enhancing PTEN, thus enhancing the immune suppression functions of Treg cells and inducing immune tolerance. *c.* PD-1/PD-L1 can promote the polarization of tumor-associated macrophages (TAM) toward the M2 phenotype, releasing large amounts of fibroblast growth factor, VEGF, TNF- α , and other cytokines to promote angiogenesis and support immune suppression, invasion, and metastasis of cancer cells, accelerating cancer progression. *d.* PD-1 on NK cells binds to PD-L1 on cancer cells, inhibiting the degranulation and cytotoxic function of NK cells, decreasing their ability to kill tumor cells, and promoting tumor immune escape. The use of PD-1 and PD-L1 inhibitors may reactivate the antitumor immune response of the above immune cells. Figure created with BioRender [87].

autoimmune conditions and infectious diseases, though the full extent of its significance is still being explored. Research on PD-L2 remains behind PD-L1, warranting further investigation to fully understand its potential in immune regulation and disease progression [92].

4.2 Cytotoxic T-lymphocyte antigen 4 (CTLA-4)

CTLA-4 is a transmembrane protein that plays a key role in regulating immune signaling by binding to ligands on the surface of APCs. It is predominantly expressed on activated T cells during the initial stages of immune activation [93]. For full T-cell activation, a costimulatory signal is necessary, which occurs when the CD28 molecule on T cells binds to CD80 (B7-1) and CD86 (B7-2) on APCs. While CTLA-4 shares a similar molecular makeup with CD28, it has a much stronger binding affinity for CD80 and CD86. This competitive binding effectively inhibits T-cell activation by preventing CD28 from engaging with these costimulatory molecules [94].

Tumor cells take advantage of this pathway by secreting TGF- β , which either promotes increased CTLA-4 expression on T cells or strengthens CTLA-4's interaction with CD80/CD86. Once CTLA-4 is activated, T-cell-mediated tumor destruction is decreased, enabling tumor cells to evade immune attack and supporting tumor progression. Furthermore, CTLA-4 operates together with other immune checkpoints, such as PD-1, creating a synergistic effect that enhances immune evasion throughout tumor development [94].

4.3 T-cell immunoglobulin and mucin-domain containing-3 (TIM-3)

TIM-3 is an immunological checkpoint receptor that suppresses the activation and proliferation of Th1 and cytotoxic T (Tc1) cells. Th1 cells, Th17 cells, and Tregs are among the CD4+ T-cell subsets that express it the most. The Fyn kinase can bind when TIM-3 interacts with its primary ligand, galectin-9, which causes the release of BAT3 (HLA-B-associated transcript 3) [95]. This cascade promotes apoptosis in Th1 and Th17 cells, thereby dampening the immune response and impairing the activation of other immune effector cells.

Additionally, by preventing the production of cytotoxic cytokines and triggering apoptosis in activated T cells, galectin-9 can interact with TIM-3 and PD-1 at the same time, thereby contributing to T-cell exhaustion [96]. Tumor cells may utilize increased TIM-3 expression, especially in monocytes, to upregulate galectin-9 on CD4+ T cells. As observed in patients with HNSCC, this process inhibits efficient antitumor immunity, resulting in insufficient tumor clearance and potentially resulting in tumor persistence and recurrence [97].

4.4 Lymphocyte activation gene-3 (LAG-3)

TILs, Tregs, and activated CD4+ and CD8+ T cells all express the class I transmembrane protein LAG-3 [98]. LAG-3 expression is significantly increased upon T cell activation, particularly in pathological conditions. It interferes with the MHC-II-CD4/TCR interaction and inhibits TCR activation by binding to major histocompatibility complex class II (MHC-II) molecules more strongly than CD4 [98, 99]. LAG-3 is mostly present on Tregs, where it interacts with MHC-II on tumors or DCs to improve their stability and immunosuppressive activity [100].

LAG-3, PD-1, and TIM-3 are among the several IRs that are co-expressed on malfunctioning or “exhausted” T cells in the TME. Poor clinical outcomes and compromised T-cell-mediated tumor suppression are frequently linked to this co-expression [101]. Notably, LAG-3 and PD-1 work in tandem to increase T-cell suppression, especially in CD8+ T cells, which leads to immunological failure in cancer and persistent infections [102]. This cooperative process is a crucial target for combination immunotherapy techniques and provides malignancies with a strong immune escape mechanism.

4.5 Glucocorticoid-induced TNFR family-related protein (GITR)

NK cells, T cells, and B cells all express glucocorticoid-induced TNFR-related protein (GITR), a type I transmembrane receptor that is a member of the tumor necrosis factor receptor superfamily (TNFRSF). Notably, it is found at lower levels on naïve and memory T cells and at higher levels on Tregs [103]. When GITR binds

to its ligand, GITRL, it triggers the nuclear factor kappa B (NF- κ B) signaling pathway, which increases CD8⁺ T-cell responses to tumor antigens and effector T-cell cytotoxicity [104].

It has been demonstrated that GITR activation short-term suppresses Tregs, hence enhancing antitumor immunity. On the other hand, Treg activation and growth may paradoxically result from prolonged or excessive GITR/GITRL pathway stimulation [105]. This change has the potential to eventually inhibit effector T cells' antitumor function, allowing tumor cells to reestablish immune evasion and perpetuate tumor growth. These dual effects highlight the complexities of GITR targeting in cancer immunotherapy, as well as the importance of dosage and timing in therapeutic applications.

5. Therapeutic approaches and clinical implications

The major clinical treatment for HNSCC continues to be open surgical resection combined with lymph node and tumor dissection. However, it frequently results in significant functional impairments that affect patients' quality of life, including changes in the physical appearance of the head and neck, as well as challenges with speech and swallowing. Radiation stomatitis is one of the side effects of radiotherapy, which uses high-energy rays [106]. The effectiveness of radiation alone is reduced for larger or more advanced tumors [107].

For advanced HNSCC, chemotherapy serves as a palliative treatment that can help reduce symptoms and lengthen survival. Common medications such as 5-fluorouracil (5-FU) and cisplatin function by hindering DNA replication and synthesis. Chemotherapy, however, has serious adverse effects, including nausea and bone marrow suppression, and long-term treatment can result in tumor cell drug resistance [108]. The primary method of early-stage treatment continues to be surgery.

Even with the utilization of multidisciplinary techniques such as chemotherapy, radiation, surgery, and other treatments, 40–60% of patients still relapse following treatment [109]. Monoclonal antibodies targeting EGFR were the first to enter clinical use in targeted therapy for HNSCC [110]. The U.S. FDA approved cetuximab, a representative medication, as the first monoclonal antibody (mAb) for targeted therapy. It inhibits the growth of cancer cells and triggers death by competitively binding to EGFR tyrosine kinase and disrupting intracellular signal transduction pathways [110]. To increase efficacy, combinations of immunotherapies or other treatments are investigated.

The development of novel immunotherapies for HNSCC has drawn increasing attention worldwide in recent years. Consequently, a variety of PD-1/PD-L1 inhibitors and immune checkpoint inhibitors (ICIs), such as monoclonal antibodies that target PD-1/PD-L1 and CTLA-4, have been used more frequently in the treatment of HNSCC (**Figure 6**). Additionally, the development of combination treatment techniques has advanced significantly, including the integration of ICIs with targeted treatments, chemotherapy, and radiotherapy [111].

5.1 Immune checkpoint inhibitor-based therapeutics (ICIs) in HNSCC

Within the TME, several immune checkpoint pathways, both IRs (PD-1, CTLA-4, LAG-3, TIM-3) and stimulatory ligands (CD40L, CD70), become dysregulated, enabling tumors to evade immune surveillance [112].

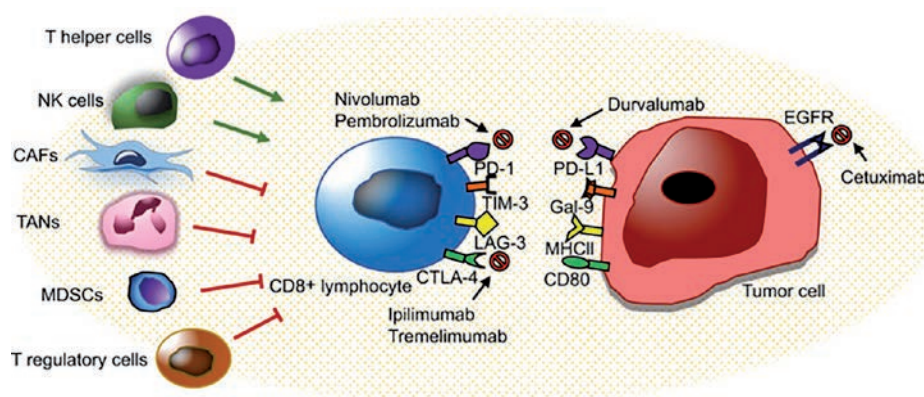


Figure 6. Immunoregulators and immunotherapeutic strategies in HNSCC. The inflammatory state of the TME can modulate CD8+ T-lymphocyte response to cancer cells. Nonmalignant cells (CAFs, TANs, MDSCs, and Tregs) in the TME have been reported to inhibit CD8+ T-lymphocyte while NK cells and Th lymphocytes both have CD8+ T-lymphocyte supportive functions. HNSCC is infiltrated with immune cells. Cetuximab, an EGFR inhibitor, was the first immune-targeted therapy to be approved in HNSCC. PD-1 immune checkpoint inhibitors, nivolumab and pembrolizumab, have recently been granted approval for use in advanced or metastatic HNSCC. Immune checkpoint inhibitors (PD-1, PD-L1, CTLA-4, TIM-3, and LAG-3) are important therapeutic targets for the treatment and prevention of HNSCC [24].

The binding of PD-1 receptors on T cells to their ligands, PD-L1 and PD-L2, often expressed by tumor or stromal cells, leads to T-cell exhaustion and diminished cytotoxic function, allowing tumor cells to escape immune-mediated destruction. ICI therapies aim to disrupt this interaction, most notably, the PD-1/PD-L1 axis, to restore T-cell activity and reinvigorate antitumor responses [113]. However, because ICIs typically block a single pathway, other immune escape routes may remain active. For this reason, combinatorial strategies involving multiple ICIs or integration with other targeted therapies are under investigation to enhance clinical efficacy.

CTLA-4, another well-established immune checkpoint molecule, negatively regulates T-cell activation by interfering with the phosphoinositide 3-kinase (PI3K) signaling cascade and the T-cell surface glycoprotein CD3 zeta chain of the TCR, thereby dampening T-cell signaling. Additional checkpoints of therapeutic interest include TIM-3, LAG-3, VISTA (V-domain Ig suppressor of T-cell activation), TIGIT (T-cell immunoglobulin and ITIM domain), and Sialic acid binding Ig-like lectin 15 (Siglec-15). These molecules have shown relevance in advanced malignancies, including melanoma, non-small cell lung cancer (NSCLC), and HNSCC [114].

Collectively, understanding the diverse roles of immune checkpoints in the TME provides a foundation for the development of more effective immunotherapies, especially in tumors like HNSCC, where immune evasion is a hallmark of disease progression.

5.2 Inhibitory antibodies against PD-1/PD-L1

By preventing PD-1 and PD-L1 from interacting, anti-PD-1/PD-L1 medications reduce immunosuppressive signaling [115].

The first-line therapy option for individuals with R/M HNSCC has historically been the EXTREME regimen, which consists of cetuximab combined with

platinum-based medications and 5-FU [116]. When the FDA in the United States authorized this regimen in 2006 for the treatment of stage III HNSCC, it was regarded as the standard treatment.

Pembrolizumab is a mAb that is highly selective against PD-1. It works by inhibiting the interaction between PD-1 and PD-L1 and PD-L2, which in turn reduces immuno-suppression. When used alone, pembrolizumab's objective response rate (ORR) for patients with high PD-L1 expression (combined positive score CPS ≥ 20) ranges from 18–22%. In patients with advanced HNSCC, pembrolizumab with chemotherapy results in a much greater ORR (about 35–45%) compared with chemotherapy alone [117].

According to the CheckMate-141 trial, patients with R/M HNSCC who received anti-PD-1 monotherapy had a greater OS rate than those receiving traditional therapies [118]. These results suggest that immunotherapy targeting the PD-1/PD-L1 pathway, either alone or in combination, can successfully reduce tumor size in certain patients. When compared to conventional therapies, pembrolizumab, when used as a first-line monotherapy for patients with high PD-L1 expression (CPS > 20), considerably extends OS in terms of survival.

Pembrolizumab and nivolumab were authorized by the FDA in 2016 as second-line therapies for R/M HNSCC. KEYNOTE-040 and CheckMate-141, two phase III clinical trials, showed that immunotherapy was superior to chemotherapy for R/M HNSCC patients [119]. According to PD-L1 expression levels, the KEYNOTE-048 phase III multicenter trial also showed that pembrolizumab, either by itself or in conjunction with chemotherapy, considerably increased OS as compared to the EXTREME regimen.

Pembrolizumab monotherapy and its combination with platinum-based chemotherapy were established as the new standard therapies for R/M HNSCC according to the findings of KEYNOTE-048 [120]. The FDA approved pembrolizumab as a first-line treatment in 2019, whether used alone or in conjunction with chemotherapy [121]. In addition to HNSCC, PD-1/PD-L1 monoclonal antibody-based combination therapy has been authorized by the FDA and the National Medical Products Administration (NMPA) as first-line treatments for melanoma, renal cell carcinoma (RCC), and NSCLC [122].

An IgG4 mAb called nivolumab, another PD-1 inhibitor, inhibits inhibitory signaling via the PD-1/PD-L1 pathway [123]. Patients with R/M HNSCC who were progressing after platinum-based chemotherapy are able to utilize it [124]. The median OS for R/M HNSCC patients treated with nivolumab after progressing during or after platinum-based chemotherapy was 7.5 months in the CheckMate-141 clinical study, while the median OS for patients treated with traditional single-agent therapies such as methotrexate, docetaxel, or cetuximab was 5.1 months [118]. Additionally, nivolumab dramatically increased the 12-month and 18-month survival rates, which were 36.0% and 26.1%, respectively, in comparison to 16.6% and 15.4% in the group receiving conventional therapy, according to the study. These findings demonstrate the potential of nivolumab as a successful treatment for advanced HNSCC.

There are a few PD-L2 inhibitor medications in clinical trials at present, but their numbers are minor, and further research is needed to determine their clinical efficacy.

Until now, the FDA has approved five monoclonal antibodies targeting the PD-1/PD-L1 immune checkpoint axis for cancer immunotherapy. Among these, atezolizumab and durvalumab specifically target PD-L1, while nivolumab, pembrolizumab, and cemiplimab are directed against PD-1. Both nivolumab and pembrolizumab are approved for the treatment of patients with R/M HNSCC who are resistant to

cisplatin-based chemotherapy [125]. Cemiplimab, on the other hand, has shown robust clinical activity and is approved for use in metastatic cutaneous squamous cell carcinoma (CSCC) [126].

The anti-PD-1/PD-L1 medications tislelizumab and sintilimab are still in the experimental stage, and research is being done to determine the best dosages and treatment schedules [127, 128]. In the meantime, phase III clinical trials are being conducted on durvalumab, an inhibitory antibody that targets PD-L1, to evaluate its safety and effectiveness as a treatment for HNSCC alone as well as in conjunction with chemotherapy, radiation, and other therapies [129]. It is anticipated to give patients with HNSCC new treatment alternatives.

5.3 Targeting CTLA-4

CTLA-4 inhibiting antibodies have demonstrated notable clinical success in the treatment of melanoma. For example, the first immune checkpoint inhibitor authorized for the treatment of melanoma was ipilimumab [130]. According to pivotal clinical research (MDX010-20), individuals treated with ipilimumab had a 3-year survival rate of 20–25%, while those receiving conventional treatments only had a rate of 10–15%. Ipilimumab increased OS in patients with advanced melanoma when used as a monotherapy [130]. Additionally, its effectiveness significantly increased when paired with PD-1 inhibitors like nivolumab, with ORR rising to 50–60%.

Apart from melanoma, ipilimumab has shown promise in combination treatments for colorectal cancer (CRC), RCC, and NSCLC, all of which have improved survival outcomes. Ipilimumab, on the other hand, is mostly utilized in combination therapies for melanoma; monotherapy is reserved for specific cases [25].

Tregs that express membrane-bound CTLA-4 contribute to the modulation of antitumor immunity in the HNSCC TME [16]. Inhibitors of CTLA-4 aid in reversing this immunosuppressive effect. However, CTLA-4 inhibitory antibody monotherapy has demonstrated poor efficacy in HNSCC, proving less effective than PD-1/PD-L1 therapies and being linked to more serious side effects (potential serious toxicity, including life-threatening colitis) [16]. Because of their encouraging outcomes in clinical studies, CTLA-4 inhibitors are commonly utilized in conjunction with PD-1/PD-L1 inhibitors for a synergistic impact, as well as with chemotherapy, radiation, and cellular therapies. Current clinical trials in patients with advanced HNSCC (NCT01860430 and NCT01935921) are diving into the effectiveness of ipilimumab when it's teamed up with cetuximab and intensity-modulated radiotherapy (IMRT). Meanwhile, there's an exciting phase I study underway aimed at exploring the safety and the best dosing of MGA271—also known as enoblituzumab. This humanized mAb directly targets CD276 (B7-H3) and is being investigated in partnership with ipilimumab, particularly for those patients who have B7-H3-expressing solid tumors, including HNSCC (NCT02381314). Another intriguing contender in the arena of anti-CTLA4 antibodies, tremelimumab, is also being put to the test. It's being studied in tandem with the anti-PD-L1 antibody durvalumab [131]. A randomized phase II clinical trial involving 267 patients with R/M HNSCC revealed some promising findings—the combination of durvalumab and tremelimumab showed not only significant OS benefits but also manageable side effects [132]. Additionally, the CheckMate 651 clinical trial (NCT02823574) provided some compelling evidence that a combination of nivolumab and ipilimumab serves as a powerful disease control agent in R/M HNSCC, boosting the median OS from 13.5 to 13.9 months when compared to the EXTREME therapy regimen [133].

5.4 PD-1/PD-L1 inhibitor combined with CTLA-4 inhibitor therapy

Navigating the intricate world of T-cell regulation is no easy feat, especially when you consider the pivotal role of the immune checkpoint protein CTLA-4, which is believed to play a crucial part in the restraint of T-cell activation. This has sparked the intriguing idea of simultaneously targeting both the PD-1 and CTLA-4 pathways—a strategy that promises a more well-rounded approach to ICI, particularly in cases where PD-L1 expression is notably absent. Adding CTLA-4 targeting to the existing therapy of nivolumab has shown promising improvements in survival rates for melanoma patients [134]. While it is true that antibodies specifically aimed at CTLA-4 show limited effectiveness as stand-alone treatments for HNSCC, researchers have delved into the potential of combining a PD-1- or PD-L1-directed antibody with anti-CTLA-4. Initial findings suggest that this combination is not only safe but could open new avenues for treatment. Two different immune checkpoint mechanisms are represented by CTLA-4 and PD-1/PD-L1 and targeting both at the same time can have a synergistic antitumor effect (**Figure 7**) [135].

Currently, agents that target PD-L1 and CTLA-4 exhibit lower activity in HNSCC compared to various other solid tumors. Certain characteristics inherent to HNSCC may negatively influence the efficacy of ICI. In cases of locoregional recurrence, challenges such as tumor bulk and hypoxia can hinder the effective infiltration of activated T cells into the tumor [136]. High levels of MDSC and M2-macrophages contribute to an immunosuppressive inflammatory environment in HNSCC [137]. Moreover, for patients who have undergone cisplatin and radiation as definitive or postoperative treatments within a curative context, the persistence of treatment-induced lymphopenia is frequently observed and correlates with diminished benefits from ICI [138]. Preclinical studies indicate that the ablation of lymph nodes impairs the efficacy of anti-PD-1 or anti-CTLA-4 antibodies in controlling tumor growth, underscoring the critical role of tumor-draining lymph nodes in the anticancer immune response [139]. The aforementioned considerations suggest that HNSCCs that have not undergone prior treatment—meaning there has been no previous surgery or radiation that could exacerbate tumor hypoxia, eliminate tumor-draining lymph nodes, or cause lasting lymphopenia—might respond more favorably to ICI.

The use of combination ICI with various coinhibitory molecules has the potential to enhance treatment responses by differentially modulating diverse cell populations within the TME [140]. In the CheckMate 651 study, which assessed the effects of combining α -PD-1 and α -CTLA-4 blockade in R/M HNSCC, there was no observed change in the ORR or OS [133]. Likewise, CheckMate 714 reported no alteration in ORR when comparing α -PD-1/ α -CTLA-4 inhibition to α -PD-1 alone in platinum-refractory R/M HNSCC [141]. The phase III EAGLE trial investigated durvalumab, an α -PD-L1 monoclonal antibody, in comparison to durvalumab combined with tremelimumab (α -CTLA-4) and standard of care (SoC) in patients with R/M HNSCC, finding no significant differences in OS across the groups relative to SoC [142].

Simultaneously, KESTREL observed no advantage in using either single-agent or combination α -PD-L1, with or without α -CTLA-4. It was noted that patients exhibiting high PD-L1 expression who received the standard of SoC demonstrated a superior ORR compared to those treated with durvalumab alone or in conjunction with tremelimumab [143].

Ipilimumab and pembrolizumab together are now being studied, and the findings have been encouraging. By improving immune activation through several methods, this dual blockade increases the efficiency of immune cells in destroying tumor cells [25, 131].

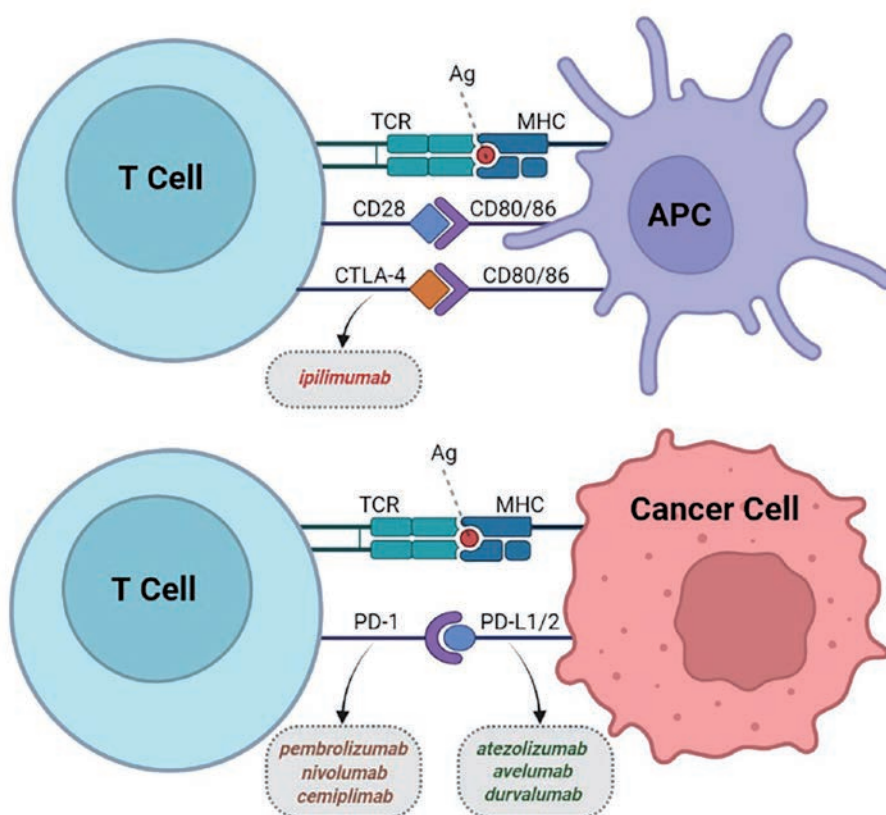


Figure 7. FDA-approved classes of immune checkpoint inhibitors (CPIs). CTLA-4 (through the interaction with its ligands B7-1/CD80 and B7-2/CD86) or PD-1 (via binding to its ligand PD-L1) triggers inhibitory signals to attenuate T-cell immune response. These T-cell receptor targets provide a rationale for the use of CPIs such as anti-CTLA-4, PD-1, and PD-L1, which are illustrated with dotted-border boxes to increase immune response and kill tumor cells. CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; PD-1/PD-L1: Programmed cell death protein-1 and its ligand-1, respectively; APC: Antigen-presenting cell; Ag: Antigen; TCR: T-cell receptor; MHC: Major histocompatibility complex. (Figure was designed with BioRender.com, <https://help.biorender.com/en/articles/3619405-how-do-i-cite-biorender>, accessed on 7 November 2022) [135].

Early clinical trials have shown that the combination of PD-1/PD-L1 and CTLA-4 inhibitors increases the ORR and prolongs patient survival when compared to the EXTREME regimen [25]. Nevertheless, concurrent use of two checkpoint inhibitors also results in more severe side effects. Therefore, it is essential to put methods for managing and minimizing treatment-related problems into place while prioritizing therapeutic efficacy.

5.5 Combinatory approaches to overcome resistance and boost tumor-specific responses

Combinatory methods are being actively researched to address resistance mechanisms and improve efficacy. Currently, tumor-related antibodies are primarily utilized in combination with chemotherapy, as their effectiveness as monotherapies is limited [144]. The efficacy of ICI therapy can be enhanced

through chemotherapy, which facilitates the release of neoantigens, alters the TME by depleting Tregs and MDSCs, and diminishes PD-L2 expression on DCs and tumor cells. Additionally, chemotherapy may stimulate the maturation of APCs and elevate MHC-I expression [145]. An example of an approved combination regimen includes the use of pemetrexed and carboplatin chemotherapy alongside pembrolizumab, which shows minimal overlapping toxicity with the chemotherapeutic agents [146].

Other combinations being investigated include the use of nivolumab and ipilimumab as standard therapy for first-line treatment of HNSCC (NCT02741570) [147]. However, it is important to note that chemotherapy may cause side effects that interfere with the mechanism of action of ICIs, for example, inhibiting the clonal expansion of effector lymphocytes. Therefore, the potential effects of chemotherapy must be carefully considered when formulating treatment strategies.

According to recent research, individuals with advanced HNSCC may have a much higher ORR and progression-free survival if pembrolizumab is combined with chemotherapy, such as cisplatin and 5-FU [148]. Induction chemotherapy with TPF (docetaxel, platinum-based medications, and 5-FU) has also been shown to increase PD-L1 expression in tumor cells and immune cells that infiltrate tumors [149]. Additionally, in locally advanced HNSCC, it raises the density of CD8⁺ T cells. A successful treatment method for HNSCC patients may involve combining induction chemotherapy with anti-PD-1/PD-L1 immunotherapy, as higher levels of PD-L1 and CD8⁺ T cells enhance the interaction between tumors and immune ICIs.

Coadministration of TLR agonists and PD-1 inhibitors has demonstrated promise in boosting antigen presentation and reactivating TAMs, hence promoting tumor-specific immune responses [150]. Similarly, it has been shown that CD8⁺ T-cell function in the TME can be restored when cetuximab and PD-1 inhibition are combined [151]. Strategies that target immune evasion pathways, such as downregulating MHC class I, have also drawn more interest. Through STAT3 activation, MEK inhibitors such as trametinib have been demonstrated to upregulate MHC class I and PD-L1 [152]. In preclinical settings, this dual strategy inhibits tumor growth and encourages CD8⁺ T-cell infiltration when paired with PD-L1 inhibition.

Radiotherapy is commonly used to treat solid tumors; more than 50% of patients receive this treatment. The advantage of radiotherapy over chemotherapy is that it minimizes systemic toxicity [153]. When combined with ICIs, radiotherapy has a synergistic effect that helps normalize tumor vasculature, enhances the expression of leukocyte adhesion molecules on endothelial cells, and stimulates the secretion of chemokines that attract CD8⁺ T cells [154].

ICI therapy is indicated for patients with R/M HNSCC who have previously responded to radiotherapy, particularly those whose immune cells have shifted to an immunosuppressive and radioresistant phenotype. A phase II study (NCT02641093) showed that the use of pembrolizumab as adjuvant radiotherapy improved survival in patients with locally advanced HNSCC [155]. In some patients, following radiotherapy may induce somatic mutations, leading to the generation of new tumor-associated antigens (TAAs) that can generate a more robust immune response against these antigens. Concomitant administration of radiotherapy with ICIs, such as nivolumab (NCT02684253 and NCT03349710), is generally safe and does not cause significant immune-related adverse reactions in HNSCC [156].

6. Conclusions

In advanced stages, when traditional therapy approaches frequently fail, HNSCC remains a major clinical problem. Multimodal strategies, such as postoperative chemoradiotherapy and neoadjuvant treatments followed by surgical resection, have shown promise in turning incurable tumors into ones that can be removed. Despite their potential, these approaches are frequently constrained by significant disadvantages, including a lower quality of life following surgery, extreme toxicity from chemotherapy and radiation, and a generally poor prognosis in later stages of the disease. The necessity for novel treatment paradigms is highlighted by the persistent concerns about metastasis and recurrence [157].

In this regard, immunotherapy has become a game-changing alternative that gives HNSCC patients new hope. In recent years, with an in-depth understanding of cancer immunology, cancer immunotherapy for HNSCC has become increasingly important. This innovative approach is changing the treatment landscape of HNSCC. Both immune monotherapy and combination therapy can reduce morbidity and prolong the survival of HNSCC patients compared with traditional treatments. However, there are still many challenges in the field of cancer immunotherapy for HNSCC, and we are still in the early stages of exploring the optimal combination of surgery, chemotherapy, radiotherapy, and immunotherapy. Therefore, challenges such as tumor immune resistance, immune escape, and immune-related side effects need to be studied to clearly understand the basic principles of immune regulatory mechanisms in HNSCC and progress in this field [16].

Clinical trials have demonstrated promising outcomes for ICIs, especially those that target the PD-1/PD-L1 and CTLA-4 pathways [158]. The therapeutic effect is still only available to a tiny percentage of patients, though. The widespread use of these treatments is severely limited by the variability of TME, the absence of reliable prognostic biomarkers, and the frequency of immune-related adverse events (irAEs).

Given that it is extremely immunosuppressive and drug-resistant, the TME in HNSCC continues to be a major challenge. It is, nevertheless, also a useful therapeutic target. The TME of HNSCC is composed of many different cell subsets that infiltrate the tumor and interact with tumor cells or each other through different networks.

They play crucial roles in mediating immune surveillance and controlling tumor growth and contribute to the immunosuppressive TME of HNSCC. A better understanding of the various factors that play key roles in HNSCC tumorigenesis and tumor growth may help identify novel targets to develop more effective immunotherapies for HNSCC patients. The development of the next generation of immunotherapy for HNSCC treatments that are not only more efficient and long-lasting but also customized to each patient's particular biology will ultimately depend on our ability to better comprehend tumor-immune interactions and overcome existing constraints.

Author details

Eleni Litsou
University Hospital of Ioannina, Greece

*Address all correspondence to: elenilitsou@gmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Chow LQM. Head and neck cancer. *The New England Journal of Medicine*. 2020;**382**(1):60-72. DOI: 10.1056/NEJMra1715715
- [2] Johnson DE, Burtneess B, Leemans CR, Lui VWY, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma. *Nature Reviews. Disease Primers*. 2020;**6**(1):92. DOI: 10.1038/s41572-020-00224-3. Erratum in *Nat Rev Dis Primers*. 2023;**9**(1):4. DOI: 10.1038/s41572-023-00418-5
- [3] Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A systematic analysis for the global burden of disease study. *JAMA Oncology*. 2017;**3**(4):524-548. DOI: 10.1001/jamaoncol.2016.5688. Erratum in *JAMA Oncol*. 2017 Mar 1;**3**(3):418. DOI: 10.1001/jamaoncol.2017.0098
- [4] Skálová A, Hyrcza MD, Leivo I. Update from the 5th edition of the World Health Organization classification of head and neck tumors: Salivary glands. *Head and Neck Pathology*. 2022;**16**(1):40-53. DOI: 10.1007/s12105-022-01420-1. Epub 2022 Mar 21
- [5] Miyauchi S, Kim SS, Pang J, Gold KA, Gutkind JS, Califano JA, et al. Immune modulation of head and neck squamous cell carcinoma and the tumor microenvironment by conventional therapeutics. *Clinical Cancer Research*. 2019;**25**(14):4211-4223. DOI: 10.1158/1078-0432.CCR-18-0871. Epub 2019 Feb 27
- [6] Lalango F, Kabagenyi F, Seguya A, Byaruhanga R, Otiti J. A descriptive study on diagnostic timelines, and factors influencing delayed diagnosis among adult head and neck cancer patients at Uganda cancer institute. *World Journal of Surgical Oncology*. 2024;**22**(1):130. DOI: 10.1186/s12957-024-03407-4
- [7] Benhamou CA, Laraqui N, Touhami M, Chekkoury A, Benchakroun Y, Samlali R, et al. Tabac et cancer du larynx: Enquête prospective à propos de 58 malades [Tobacco and cancer of the larynx: A prospective survey of 58 patients]. *Rev Laryngol Otol Rhinol (Bord)*. 1992;**113**(4):285-288. French
- [8] Chaturvedi AK, Anderson WF, Lortet-Tieulent J, Curado MP, Ferlay J, Franceschi S, et al. Worldwide trends in incidence rates for oral cavity and oropharyngeal cancers. *Journal of Clinical Oncology*. 2013;**31**(36):4550-4559. DOI: 10.1200/JCO.2013.50.3870. Epub 2013 Nov 18
- [9] Carvalho AL, Nishimoto IN, Califano JA, Kowalski LP. Trends in incidence and prognosis for head and neck cancer in the United States: A site-specific analysis of the SEER database. *International Journal of Cancer*. 2005;**114**(5):806-816. DOI: 10.1002/ijc.20740
- [10] Tiwana MS, Wu J, Hay J, Wong F, Cheung W, Olson RA. 25-year survival outcomes for squamous cell carcinomas of the head and neck: Population-based outcomes from a Canadian province. *Oral Oncology*. 2014;**50**(7):651-656. DOI: 10.1016/j.oraloncology.2014.03.009. Epub 2014 Apr 14
- [11] D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral

sexual behaviors associated with prevalent oral human papillomavirus infection. *The Journal of Infectious Diseases*. 2009;**199**(9):1263-1269. DOI: 10.1086/597755

[12] Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. *Journal of the National Cancer Institute*. 2008;**100**(4):261-269. DOI: 10.1093/jnci/djn011. Epub 2008 Feb 12

[13] Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature*. 2012;**489**(7417):519-525. DOI: 10.1038/nature11404. Epub 2012 Sep 9. Erratum in *Nature*. 2012 Nov 8;491(7423):288. Rogers, Kristen [corrected to Rodgers, Kristen]

[14] Ghosh S, Shah PA, Johnson FM. Novel systemic treatment modalities including immunotherapy and molecular targeted therapy for recurrent and metastatic head and neck squamous cell carcinoma. *International Journal of Molecular Sciences*. 2022;**23**(14):7889. DOI: 10.3390/ijms23147889

[15] Jiang B, Elakashif A, Coulter JA, Dunne NJ, McCarthy HO. Immunotherapy for HPV negative head and neck squamous cell carcinoma. *Biochimica Et Biophysica Acta. Reviews on Cancer*. 2024;**1879**(5):189138. DOI: 10.1016/j.bbcan.2024.189138. Epub 2024 Jun 16

[16] Wang CW, Biswas PK, Islam A, Chen MK, Chueh PJ. The use of immune regulation in treating head and neck squamous cell carcinoma (HNSCC). *Cells*. 2024;**13**(5):413. DOI: 10.3390/cells13050413

[17] Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science*. 2015;**348**(6230):74-80. DOI: 10.1126/science.aaa6204

[18] Li Y, Zhao L, Li XF. Hypoxia and the tumor microenvironment. *Technology in Cancer Research & Treatment*. 2021;**20**:15330338211036304. DOI: 1177/15330338211036304

[19] Anderson NM, Simon MC. The tumor microenvironment. *Current Biology*. 2020;**30**(16):R921-R925. DOI: 10.1016/j.cub.2020.06.081

[20] Alasvand N, Urbanska AM, Rahmati M, Maryam S, Selcan Gungor-Ozkerim P, Sefat F, et al. Therapeutic nanoparticles for targeted delivery of anticancer drugs. In: Grumezescu AM, editor. *Multifunctional Systems for Combined Delivery, Biosensing and Diagnostics*. Amsterdam: Elsevier; 2017. pp. 245-259. ISBN 9780323527255. DOI: 10.1016/B978-0-323-52725-5.00013-7

[21] Danhier F, Feron O, Pr  at V. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *Journal of Controlled Release*. 2010;**148**(2):135-146. DOI: 10.1016/j.jconrel.2010.08.027. Epub 2010 Aug 24

[22] Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: A systematic review. *Molecular Cancer*. 2019;**18**(1):63. DOI: 10.1186/s12943-019-0983-5

[23] Ramos RN, Amano MT, Paes Leme AF, Fox JW, de Oliveira AK. Editorial: Tumor microenvironment (TME) and tumor immune microenvironment (TIME): New

perspectives for prognosis and therapy. *Frontiers in Cell and Development Biology*. 2022;**10**:971275. DOI: 10.3389/fcell.2022.971275

[24] Elmusrati A, Wang J, Wang CY. Tumor microenvironment and immune evasion in head and neck squamous cell carcinoma. *International Journal of Oral Science*. 2021;**13**(1):24. DOI: 10.1038/s41368-021-00131-7

[25] Zhang Z, Meng X, Han L. Application and Perspectives of Immunotherapy in Head and Neck Squamous Cell Carcinoma. *Immunology*. 21 May 2025. DOI: 10.1111/imm.13951. Epub ahead of print

[26] Jorgovanovic D, Song M, Wang L, Zhang Y. Roles of IFN- γ in tumor progression and regression: A review. *Biomarker Research*. 2020;**8**:49. DOI: 10.1186/s40364-020-00228-x

[27] Charles A, Janeway J, Travers P, Walport M, Shlomchik MJ. The major histocompatibility complex and its functions. In: *Immunobiology: The Immune System in Health and Disease*. 5th ed. New York: Garland Science; 2001. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK27156/>

[28] Zhang N, Bevan MJ. CD8(+) T cells: Foot soldiers of the immune system. *Immunity*. 2011;**35**(2):161-168. DOI: 10.1016/j.immuni.2011.07.010

[29] Alspach E, Lussier DM, Schreiber RD. Interferon γ and its important roles in promoting and inhibiting spontaneous and therapeutic cancer immunity. *Cold Spring Harbor Perspectives in Biology*. 2019;**11**(3):a028480. DOI: 10.1101/cshperspect.a028480

[30] Wahbi HS, Manadili A. Immunoexpression of tumor

infiltrating lymphocytes (TILs) CD4 + and CD8 + in Oral squamous cell carcinoma (OSCC) in correlations with Clinicopathological characteristics and prognosis. *Asian Pacific Journal of Cancer Prevention*. 2022;**23**(12):4177-4183. DOI: 10.31557/APJCP.2022.23.12.4177

[31] de Ruiter EJ, Ooft ML, Devriese LA, Willems SM. The prognostic role of tumor infiltrating T-lymphocytes in squamous cell carcinoma of the head and neck: A systematic review and meta-analysis. *Oncoimmunology*. 2017;**6**(11):e1356148. DOI: 10.1080/2162402X.2017.1356148

[32] Sawa-Wejksza K, Kandeferszyszeń M. Tumor-associated macrophages as target for antitumor therapy. *Archivum Immunologiae et Therapiae Experimentalis (Warsz)*. 2018;**66**(2):97-111. DOI: 10.1007/s00005-017-0480-8. Epub 2017 Jun 28

[33] Sun H, Miao C, Liu W, Qiao X, Yang W, Li L, et al. TGF- β 1/T β RII/Smad3 signaling pathway promotes VEGF expression in oral squamous cell carcinoma tumor-associated macrophages. *Biochemical and Biophysical Research Communications*. 2018;**497**(2):583-590. DOI: 10.1016/j.bbrc.2018.02.104. Epub 2018 Feb 17

[34] Greene S, Robbins Y, Mydlarz WK, Huynh AP, Schmitt NC, Friedman J, et al. Inhibition of MDSC trafficking with SX-682, a CXCR1/2 inhibitor, enhances NK-cell immunotherapy in head and neck cancer models. *Clinical Cancer Research*. 2020;**26**(6):1420-1431. DOI: 10.1158/1078-0432.CCR-19-2625. Epub 2019 Dec 17

[35] Gao L, Zhang W, Zhong WQ, Liu ZJ, Li HM, Yu ZL, et al. Tumor associated macrophages induce epithelial to

mesenchymal transition via the EGFR/ERK1/2 pathway in head and neck squamous cell carcinoma. *Oncology Reports*. 2018;**40**(5):2558-2572. DOI: 10.3892/or.2018.6657. Epub 2018 Aug 17

[36] Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Research*. 2006;**66**(2):605-612. DOI: 10.1158/0008-5472.CAN-05-4005

[37] Laoui D, Movahedi K, Van Overmeire E, Van den Bossche J, Schouppe E, Mommer C, et al. Tumor-associated macrophages in breast cancer: Distinct subsets, distinct functions. *The International Journal of Developmental Biology*. 2011;**55**(7-9):861-867. DOI: 10.1387/ijdb.113371dl

[38] Hartley GP, Chow L, Ammons DT, Wheat WH, Dow SW. Programmed cell death ligand 1 (PD-L1) signaling regulates macrophage proliferation and activation. *Cancer Immunology Research*. 2018;**6**(10):1260-1273. DOI: 10.1158/2326-6066.CIR-17-0537. Epub 2018 Jul 16

[39] Mattox AK, Lee J, Westra WH, Pierce RH, Ghossein R, Faquin WC, et al. PD-1 expression in head and neck squamous cell carcinomas derives primarily from functionally Anergic CD4⁺ TILs in the presence of PD-L1⁺ TAMs. *Cancer Research*. 2017;**77**(22):6365-6374. DOI: 10.1158/0008-5472.CAN-16-3453. Epub 2017 Sep 25

[40] Hirai M, Kitahara H, Kobayashi Y, Kato K, Bou-Gharios G, Nakamura H, et al. Regulation of PD-L1 expression in a high-grade invasive human oral squamous cell carcinoma microenvironment. *International Journal of Oncology*. 2017;**50**(1):41-48.

DOI: 10.3892/ijo.2016.3785. Epub 2016 Dec 2

[41] Jiang C, Yuan F, Wang J, Wu L. Oral squamous cell carcinoma suppressed antitumor immunity through induction of PD-L1 expression on tumor-associated macrophages. *Immunobiology*. 2017;**222**(4):651-657. DOI: 10.1016/j.imbio.2016.12.002. Epub 2016 Dec 14

[42] Kubota K, Moriyama M, Furukawa S, Rafiul HASM, Maruse Y, Jinno T, et al. CD163⁺CD204⁺ tumor-associated macrophages contribute to T cell regulation via interleukin-10 and PD-L1 production in oral squamous cell carcinoma. *Scientific Reports*. 2017;**7**(1):1755. DOI: 10.1038/s41598-017-01661-z

[43] Lyford-Pike S, Peng S, Young GD, Taube JM, Westra WH, Akpeng B, et al. Evidence for a role of the PD-1: PD-L1 pathway in immune resistance of HPV-associated head and neck squamous cell carcinoma. *Cancer Research*. 2013;**73**(6):1733-1741. DOI: 10.1158/0008-5472.CAN-12-2384. Epub 2013 Jan 3

[44] Baruah P, Bullenkamp J, Wilson POG, Lee M, Kaski JC, Dumitriu IE. TLR9 mediated tumor-stroma interactions in human papilloma virus (HPV)-positive head and neck squamous cell carcinoma up-regulate PD-L1 and PD-L2. *Frontiers in Immunology*. 2019;**10**:1644. DOI: 10.3389/fimmu.2019.01644

[45] Prince ME, Sivanandan R, Kaczorowski A, Wolf GT, Kaplan MJ, Dalerba P, et al. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proceedings of the National Academy of Sciences*

of the United States of America.
2007;**104**(3):973-978. DOI: 10.1073/
pnas.0610117104. Epub 2007 Jan 8

[46] Lee Y, Shin JH, Longmire M, Wang H, Kohrt HE, Chang HY, et al. CD44+ cells in head and neck squamous cell carcinoma suppress T-cell-mediated immunity by selective constitutive and inducible expression of PD-L1. *Clinical Cancer Research*. 2016;**22**(14):3571-3581. DOI: 10.1158/1078-0432.CCR-15-2665. Epub 2016 Feb 10

[47] Morvan MG, Lanier LL. NK cells and cancer: You can teach innate cells new tricks. *Nature Reviews. Cancer*. 2016;**16**(1):7-19. DOI: 10.1038/nrc.2015.5

[48] Zhang Y, Xie J, Wu H, Huang J, Zheng D, Wang S, et al. NK cell-based immunotherapy against oral squamous cell carcinoma. *Frontiers in Immunology*. 2024;**15**:1440764. DOI: 10.3389/fimmu.2024.1440764

[49] Mandal R, Şenbabaoğlu Y, Desrichard A, Havel JJ, Dalin MG, Riaz N, et al. The head and neck cancer immune landscape and its immunotherapeutic implications. *JCI Insight*. 2016;**1**(17):e89829. DOI: 10.1172/jci.insight.89829

[50] Habif G, Crinier A, André P, Vivier E, Narni-Mancinelli E. Targeting natural killer cells in solid tumors. *Cellular & Molecular Immunology*. 2019;**16**(5):415-422. DOI: 10.1038/s41423-019-0224-2. Epub 2019 Mar 25

[51] André P, Denis C, Soulas C, Bourbon-Caillet C, Lopez J, Arnoux T, et al. Anti-NKG2A mAb is a checkpoint inhibitor that promotes anti-tumor immunity by unleashing both T and NK

cells. *Cell*. 2018;**175**(7):1731-1743.e13. DOI: 10.1016/j.cell.2018.10.014. Epub 2018 Nov 29

[52] Wagner S, Wittekindt C, Reuschenbach M, Hennig B, Thevarajah M, Würdemann N, et al. CD56-positive lymphocyte infiltration in relation to human papillomavirus association and prognostic significance in oropharyngeal squamous cell carcinoma. *International Journal of Cancer*. 2016;**138**(9):2263-2273. DOI: 10.1002/ijc.29962. Epub 2016 Jan 8

[53] Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nature Communications*. 2016;**7**:12150. DOI: 10.1038/ncomms12150

[54] Alban TJ, Alvarado AG, Sorensen MD, Bayik D, Volovetz J, Serbinowski E, et al. Global immune fingerprinting in glioblastoma patient peripheral blood reveals immune-suppression signatures associated with prognosis. *JCI Insight*. 2018;**3**(21):e122264. DOI: 10.1172/jci.insight.122264

[55] Younos IH, Dafferner AJ, Gulen D, Britton HC, Talmadge JE. Tumor regulation of myeloid-derived suppressor cell proliferation and trafficking. *International Immunopharmacology*. 2012;**13**(3):245-256. DOI: 10.1016/j.intimp.2012.05.002. Epub 2012 May 17

[56] Ostrand-Rosenberg S, Sinha P. Myeloid-derived suppressor cells: Linking inflammation and cancer. *Journal of Immunology*. 2009;**182**(8):4499-4506. DOI: 10.4049/jimmunol.0802740

- [57] Fleming V, Hu X, Weber R, Nagibin V, Groth C, Altevogt P, et al. Targeting myeloid-derived suppressor cells to bypass tumor-induced immunosuppression. *Frontiers in Immunology*. 2018;**9**:398. DOI: 10.3389/fimmu.2018.00398
- [58] Umansky V, Blattner C, Gebhardt C, Utikal J. The role of myeloid-derived suppressor cells (MDSC) in cancer progression. *Vaccine*. 2016;**4**(4):36. DOI: 10.3390/vaccines4040036
- [59] Parker KH, Beury DW, Ostrand-Rosenberg S. Myeloid-derived suppressor cells: Critical cells driving immune suppression in the tumor microenvironment. *Advances in Cancer Research*. 2015;**128**:95-139. DOI: 10.1016/bs.acr.2015.04.002. Epub 2015 May 12
- [60] Weber R, Fleming V, Hu X, Nagibin V, Groth C, Altevogt P, et al. Myeloid-derived suppressor cells hinder the anti-cancer activity of immune checkpoint inhibitors. *Frontiers in Immunology*. 2018;**9**:1310. DOI: 10.3389/fimmu.2018.01310
- [61] Chinn IK, Blackburn CC, Manley NR, Sempowski GD. Changes in primary lymphoid organs with aging. *Seminars in Immunology*. 2012;**24**(5):309-320. DOI: 10.1016/j.smim.2012.04.005. Epub 2012 May 2
- [62] Zhao Y, Du J, Shen X. Targeting myeloid-derived suppressor cells in tumor immunotherapy: Current, future and beyond. *Frontiers in Immunology*. 2023;**14**:1157537. DOI: 10.3389/fimmu.2023.1157537
- [63] Liu T, Zhou L, Li D, Andl T, Zhang Y. Cancer-associated fibroblasts build and secure the tumor microenvironment. *Frontiers in Cell and Development Biology*. 2019;**7**:60. DOI: 10.3389/fcell.2019.00060
- [64] Elmusrati AA, Pilborough AE, Khurram SA, Lambert DW. Cancer-associated fibroblasts promote bone invasion in oral squamous cell carcinoma. *British Journal of Cancer*. 2017;**117**(6):867-875. DOI: 10.1038/bjc.2017.239. Epub 2017 Jul 25
- [65] Takahashi H, Sakakura K, Kawabata-Iwakawa R, Rokudai S, Toyoda M, Nishiyama M, et al. Immunosuppressive activity of cancer-associated fibroblasts in head and neck squamous cell carcinoma. *Cancer Immunology, Immunotherapy*. 2015;**64**(11):1407-1417. DOI: 10.1007/s00262-015-1742-0. Epub 2015 Jul 23
- [66] Cho YA, Yoon HJ, Lee JI, Hong SP, Hong SD. Relationship between the expressions of PD-L1 and tumor-infiltrating lymphocytes in oral squamous cell carcinoma. *Oral Oncology*. 2011;**47**(12):1148-1153. DOI: 10.1016/j.oraloncology.2011.08.007. Epub 2011 Sep 10
- [67] Kang JH, Jung MY, Choudhury M, Leof EB. Transforming growth factor beta induces fibroblasts to express and release the immunomodulatory protein PD-L1 into extracellular vesicles. *The FASEB Journal*. 2020;**34**(2):2213-2226. DOI: 10.1096/fj.201902354R. Epub 2019 Dec 12
- [68] Qiao XW, Jiang J, Pang X, Huang MC, Tang YJ, Liang XH, et al. The evolving landscape of PD-1/PD-L1 pathway in head and neck cancer. *Frontiers in Immunology*. 2020;**11**:1721. DOI: 10.3389/fimmu.2020.01721
- [69] Nisar S, Yousuf P, Masoodi T, Wani NA, Hashem S, Singh M, et al.

Chemokine-cytokine networks in the head and neck tumor microenvironment. *International Journal of Molecular Sciences*. 2021;**22**(9):4584. DOI: 10.3390/ijms22094584

[70] Pang X, Tang YL, Liang XH. Transforming growth factor- β signaling in head and neck squamous cell carcinoma: Insights into cellular responses. *Oncology Letters*. 2018;**16**(4):4799-4806. DOI: 10.3892/ol.2018.9319. Epub 2018 Aug 17

[71] Ma H, Yang W, Zhang L, Liu S, Zhao M, Zhou G, et al. Interferon- α promotes immunosuppression through IFNAR1/STAT1 signalling in head and neck squamous cell carcinoma. *British Journal of Cancer*. 2019;**120**(3):317-330. DOI: 10.1038/s41416-018-0352-y. Epub 2018 Dec 17

[72] Geiger JL, Grandis JR, Bauman JE. The STAT3 pathway as a therapeutic target in head and neck cancer: Barriers and innovations. *Oral Oncology*. 2016;**56**:84-92. DOI: 10.1016/j.oraloncology.2015.11.022. Epub 2015 Dec 28

[73] Ma SR, Deng WW, Liu JF, Mao L, Yu GT, Bu LL, et al. Blockade of adenosine A2A receptor enhances CD8⁺ T cells response and decreases regulatory T cells in head and neck squamous cell carcinoma. *Molecular Cancer*. 2017;**16**(1):99. DOI: 10.1186/s12943-017-0665-0

[74] Kansy BA, Concha-Benavente F, Srivastava RM, Jie HB, Shayan G, Lei Y, et al. PD-1 status in CD8⁺ T cells associates with survival and anti-PD-1 therapeutic outcomes in head and neck cancer. *Cancer Research*. 2017;**77**(22):6353-6364. DOI: 10.1158/0008-5472.CAN-16-3167. Epub 2017 Sep 13

[75] Allen CT, Clavijo PE, Van Waes C, Chen Z. Anti-tumor immunity in head and neck cancer: Understanding the evidence, how tumors escape and immunotherapeutic approaches. *Cancers (Basel)*. 2015;**7**(4):2397-2414. DOI: 10.3390/cancers7040900

[76] Komohara Y, Jinushi M, Takeya M. Clinical significance of macrophage heterogeneity in human malignant tumors. *Cancer Science*. 2014;**105**(1):1-8. DOI: 10.1111/cas.12314. Epub 2013 Nov 29

[77] Brooks JM, Menezes AN, Ibrahim M, Archer L, Lal N, Bagnall CJ, et al. Development and validation of a combined hypoxia and immune prognostic classifier for head and neck cancer. *Clinical Cancer Research*. 2019;**25**(17):5315-5328. DOI: 10.1158/1078-0432.CCR-18-3314. Epub 2019 Jun 10

[78] Coliat P, Ramolu L, Jégu J, Gaidon C, Jung AC, Pencreach E. Constitutive or induced HIF-2 addiction is involved in resistance to anti-EGFR treatment and radiation therapy in HNSCC. *Cancers (Basel)*. 2019;**11**(10):1607. DOI: 10.3390/cancers11101607

[79] Mirghani H, Amen F, Moreau F, Guigay J, Hartl DM, Lacau SGJ. Oropharyngeal cancers: Relationship between epidermal growth factor receptor alterations and human papillomavirus status. *European Journal of Cancer*. 2014;**50**(6):1100-1111. DOI: 10.1016/j.ejca.2013.12.018. Epub 2014 Jan 11

[80] Xu S, Wang H, Zhu Y, Han Y, Liu L, Zhang X, et al. Stabilization of EREG via STT3B-mediated N-glycosylation is critical for PDL1 upregulation and immune evasion in head and neck squamous cell

carcinoma. *International Journal of Oral Science*. 2024;**16**(1):47. DOI: 10.1038/s41368-024-00311-1

[81] Xavier FCA, Silva JC, Rodini CO, Rodrigues MFSD. Mechanisms of immune evasion by head and neck cancer stem cells. *Frontiers in Oral Health*. 2022;**3**:957310. DOI: 10.3389/froh.2022.957310

[82] Kondoh N, Mizuno-Kamiya M. The role of immune modulatory cytokines in the tumor microenvironments of head and neck squamous cell carcinomas. *Cancers (Basel)*. 2022;**14**(12):2884. DOI: 10.3390/cancers14122884

[83] Ai L, Chen J, Yan H, He Q, Luo P, Xu Z, et al. Research status and outlook of PD-1/PD-L1 inhibitors for cancer therapy. *Drug Design, Development and Therapy*. 2020;**14**:3625-3649. DOI: 10.2147/DDDT.S267433

[84] Munari E, Mariotti FR, Quatrini L, Bertoglio P, Tumino N, Vacca P, et al. PD-1/PD-L1 in cancer: Pathophysiological, diagnostic and therapeutic aspects. *International Journal of Molecular Sciences*. 2021;**22**(10):5123. DOI: 10.3390/ijms22105123

[85] Liu X, Yang L, Tan X. PD-1/PD-L1 pathway: A double-edged sword in periodontitis. *Biomedicine & Pharmacotherapy*. 2023;**159**:114215. DOI: 10.1016/j.biopha.2023.114215. Epub 2023 Jan 9

[86] Ortega MA, Boaru DL, De Leon-Oliva D, Fraile-Martinez O, García-Montero C, Rios L, et al. PD-1/PD-L1 axis: Implications in immune regulation, cancer progression, and translational applications. *Journal*

of Molecular Medicine (Berlin, Germany). 2024;**102**(8):987-1000. DOI: 10.1007/s00109-024-02463-3. Epub 2024 Jun 27

[87] Lin X, Kang K, Chen P, Zeng Z, Li G, Xiong W, et al. Regulatory mechanisms of PD-1/PD-L1 in cancers. *Molecular Cancer*. 2024;**23**(1):108. DOI: 10.1186/s12943-024-02023-w

[88] Schulz D, Feulner L, Santos Rubenich D, Heimer S, Rohrmüller S, Reinders Y, et al. Subcellular localization of PD-L1 and cell-cycle-dependent expression of nuclear PD-L1 variants: Implications for head and neck cancer cell functions and therapeutic efficacy. *Molecular Oncology*. 2024;**18**(2):431-452. DOI: 10.1002/1878-0261.13567. Epub 2023 Dec 26

[89] Paderno A, Petrelli F, Lorini L, Capriotti V, Gurizzan C, Bossi P. The predictive role of PD-L1 in head and neck cancer: A systematic review and meta-analysis. *Oral Oncology*. 2024;**153**:106799. DOI: 10.1016/j.oraloncology.2024.106799. Epub 2024 May 9

[90] Yearley JH, Gibson C, Yu N, Moon C, Murphy E, Juco J, et al. PD-L2 expression in human tumors: Relevance to anti-PD-1 therapy in cancer. *Clinical Cancer Research*. 2017;**23**(12):3158-3167. DOI: 10.1158/1078-0432.CCR-16-1761

[91] Zhang J, Liu Y, Xia L, Zhen J, Gao J, Atsushi T. Constructing heterogeneous single-cell landscape and identifying microenvironment molecular characteristics of primary and lymphatic metastatic head and neck squamous cell carcinoma. *Computers in Biology and Medicine*. 2023;**165**:107459. DOI: 10.1016/j.

compbiomed.2023.107459. Epub 2023 Sep 9

[92] Pai SI, Zandberg DP, Strome SE. The role of antagonists of the PD-1: PD-L1/PD-L2 axis in head and neck cancer treatment. *Oral Oncology*. 2016;**61**:152-158. DOI: 10.1016/j.oraloncology.2016.08.001. Epub 2016 Aug 5

[93] Wang S, Wu ZZ, Zhu SW, Wan SC, Zhang MJ, Zhang BX, et al. CTLA-4 blockade induces tumor pyroptosis via CD8⁺ T cells in head and neck squamous cell carcinoma. *Molecular Therapy*. 2023;**31**(7):2154-2168. DOI: 10.1016/j.ymthe.2023.02.023. Epub 2023 Mar 3

[94] Miniuk M, Reszeć-Giełazyn J, Bortnik P, Borsukiewicz A, Mroczek A. Novel predictive biomarkers in the head and neck squamous cell carcinoma (HNSCC). *Journal of Clinical Medicine*. 2024;**13**(19):5876. DOI: 10.3390/jcm13195876

[95] Liu Z, McMichael EL, Shayan G, Li J, Chen K, Srivastava R, et al. Novel effector phenotype of Tim-3⁺ regulatory T cells leads to enhanced suppressive function in head and neck cancer patients. *Clinical Cancer Research*. 2018;**24**(18):4529-4538. DOI: 10.1158/1078-0432.CCR-17-1350. Epub 2018 Apr 30

[96] Dong J, Cheng L, Zhao M, Pan X, Feng Z, Wang D. Tim-3-expressing macrophages are functionally suppressed and expanded in oral squamous cell carcinoma due to virus-induced Gal-9 expression. *Tumour Biology*. 2017;**39**(5):1010428317701651. DOI: 10.1177/1010428317701651

[97] Yang J, Xiang J, Zhu M, Zhao Y, Zhou L, Hu B, et al. Down-regulation

of HLA-B-associated transcript 3 impairs the tumoricidal effect of natural killer cells through promoting the T cell immunoglobulin and mucin domain-containing-3 signaling in a mouse head and neck squamous cell carcinoma model. *Immunobiology*. 2022;**227**(1):152127. DOI: 10.1016/j.imbio.2021.152127. Epub 2021 Jul 30

[98] Deng WW, Mao L, Yu GT, Bu LL, Ma SR, Liu B, et al. LAG-3 confers poor prognosis and its blockade reshapes antitumor response in head and neck squamous cell carcinoma. *Oncoimmunology*. 2016;**5**(11):e1239005. DOI: 10.1080/2162402X.2016.1239005

[99] Horikawa M, Masuda K, Takahashi H, Tada H, Tomidokoro Y, Motegi M, et al. Tumor antigen-specific interleukin-10-producing T-cell response in patients with head and neck squamous cell carcinoma. *Oncology Letters*. 2024;**28**(4):456. DOI: 10.3892/ol.2024.14589

[100] Mei Z, Huang J, Qiao B, Lam AK. Immune checkpoint pathways in immunotherapy for head and neck squamous cell carcinoma. *International Journal of Oral Science*. 2020;**12**(1):16. DOI: 10.1038/s41368-020-0084-8

[101] Succaria F, Kvistborg P, Stein JE, Engle EL, McMiller TL, Rooper LM, et al. Characterization of the tumor immune microenvironment in human papillomavirus-positive and -negative head and neck squamous cell carcinomas. *Cancer Immunology, Immunotherapy*. 2021;**70**(5):1227-1237. DOI: 10.1007/s00262-020-02747-w. Epub 2020 Oct 30

[102] Gavrielatou N, Vathiotis I, Aung TN, Shafi S, Burela S,

Fernandez AI, et al. Digital spatial profiling links Beta-2-microglobulin expression with immune checkpoint blockade outcomes in head and neck squamous cell carcinoma. *Cancer Research Communications*. 2023;**3**(4):558-563. DOI: 10.1158/2767-9764.CRC-22-0299

[103] Charap AJ, Enokida T, Brody R, Sfakianos J, Miles B, Bhardwaj N, et al. Landscape of natural killer cell activity in head and neck squamous cell carcinoma. *Journal for Immunotherapy of Cancer*. 2020;**8**(2):e001523. DOI: 10.1136/jitc-2020-001523

[104] Ronchetti S, Ricci E, Petrillo MG, Cari L, Migliorati G, Nocentini G, et al. Glucocorticoid-induced tumour necrosis factor receptor-related protein: A key marker of functional regulatory T cells. *Journal of Immunology Research*. 2015;**2015**:171520. DOI: 10.1155/2015/171520. Epub 2015 Apr 15

[105] Tian J, Zhang B, Rui K, Wang S. The role of GITR/GITRL interaction in autoimmune diseases. *Frontiers in Immunology*. 2020;**11**:588682. DOI: 10.3389/fimmu.2020.588682

[106] Zhou L. The application of induction chemotherapy in the treatment of locally advanced head and neck squamous cell carcinoma. *Zhonghua Yi Xue Za Zhi*. 2024;**104**(26):2373-2377. Chinese. DOI: 10.3760/cma.j.cn112137-20231011-00707

[107] Patin EC, Nenclares P, Chan Wah Hak C, Dillon MT, Patrikeev A, McLaughlin M, et al. Sculpting the tumour microenvironment by combining radiotherapy and ATR inhibition for curative-intent adjuvant immunotherapy. *Nature Communications*.

2024;**15**(1):6923. DOI: 10.1038/s41467-024-51236-6

[108] Sheykhbahaei N, Tameemi AHA, Koopaie M. Effect of short-term fasting on the cisplatin activity in human oral squamous cell carcinoma cell line HN5 and chemotherapy side effects. *BMC Cancer*. 2024;**24**(1):989. DOI: 10.1186/s12885-024-12752-2

[109] Fishman D, Choe J. Immunotherapy in head and neck cancer: Treatment paradigms, future directions, and questions. *Surgical Oncology Clinics of North America*. 2024;**33**(4):605-615. DOI: 10.1016/j.soc.2024.04.001. Epub 2024 May 10

[110] Kang JJ, Ko A, Kil SH, Mallen-St Clair J, Shin DS, Wang MB, et al. EGFR pathway targeting drugs in head and neck cancer in the era of immunotherapy. *Biochimica Et Biophysica Acta. Reviews on Cancer*. 1878;**2023**(1):188827. DOI: 10.1016/j.bbcan.2022.188827 E. Epub 2022 Oct 27

[111] Farlow JL, Brenner JC, Lei YL, Chinn SB. Immune deserts in head and neck squamous cell carcinoma: A review of challenges and opportunities for modulating the tumor immune microenvironment. *Oral Oncology*. 2021;**120**:105420. DOI: 10.1016/j.oraloncology.2021.105420. Epub 2021 Jul 1

[112] Goetz JW, Rabinowits G, Kalman N, Villa A. A review of immunotherapy for head and neck cancer. *Journal of Dental Research*. 2024;**103**(12):1185-1196. DOI: 10.1177/00220345241271992. Epub 2024 Oct 6

[113] Song P, Yan X, Jiang Y, Ye Y, Wang J, Han P. The research progress of PD-1/L1 inhibitors application in the treatment of head and neck squamous cell

carcinoma. *Lin Chuang Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*. 2022;**36**(4):315-320. Chinese. DOI: 10.13201/j.issn.2096-7993.2022.04.017

[114] Mestiri S, El-Ella DMA, Fernandes Q, Bedhiafi T, Almoghrabi S, Akbar S, et al. The dynamic role of immune checkpoint molecules in diagnosis, prognosis, and treatment of head and neck cancers. *Biomedicine & Pharmacotherapy*. 2024;**171**:116095. DOI: 10.1016/j.biopha.2023.116095. Epub 2024 Jan 6

[115] Idel C, Ribbat-Idel J, Bernuth A VON, Fleckner J, Rades D, Kirfel J, et al. Correlation of intra-tumoral and peripheral PD-1/PD-L1 immunity in head and neck cancer. *Anticancer Research*. 2023;**43**(12):5349-5358. DOI: 10.21873/anticancer.16738

[116] Falco A, Leiva M, Blanco A, Cefarelli G, Rodriguez A, Melo J, et al. First-line cisplatin, docetaxel, and cetuximab for patients with recurrent or metastatic head and neck cancer: A multicenter cohort study. *World J Clin Oncol*. 2022;**13**(2):147-158. DOI: 10.5306/wjco.v13.i2.147

[117] Silva V, Matos C. Recent updates in the therapeutic uses of Pembrolizumab: A brief narrative review. *Clinical & Translational Oncology*. 2024;**26**(10):2431-2443. DOI: 10.1007/s12094-024-03491-8. Epub 2024 Apr 24

[118] Gillison ML, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, et al. CheckMate 141: 1-year update and subgroup analysis of Nivolumab as first-line therapy in patients with recurrent/metastatic head and neck cancer. *The Oncologist*. 2018;**23**(9):1079-1082. DOI: 10.1634/theoncologist.2017-0674. Epub 2018 Jun 4

[119] Huang Y, Lan Y, Zhang Z, Xiao X, Huang T. An update on the immunotherapy for oropharyngeal squamous cell carcinoma. *Frontiers in Oncology*. 2022;**12**:800315. DOI: 10.3389/fonc.2022.800315

[120] Seiwert TY, Burtneess B, Mehra R, Weiss J, Berger R, Eder JP, et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): An open-label, multicentre, phase 1b trial. *The Lancet Oncology*. 2016;**17**(7):956-965. DOI: 10.1016/S1470-2045(16)30066-3. Epub 2016 May 27

[121] Pai-Scherf L, Blumenthal GM, Li H, Subramaniam S, Mishra-Kalyani PS, He K, et al. FDA approval summary: Pembrolizumab for treatment of metastatic non-small cell lung cancer: First-line therapy and beyond. *The Oncologist*. 2017;**22**(11):1392-1399. DOI: 10.1634/theoncologist.2017-0078. Epub 2017 Aug 23

[122] Lin J, Xue M, Gao M, Yu P, Han S. Toripalimab-induced Dermatomyositis in a patient with metastatic melanoma. *Dermatology and Therapy*. 2020;**10**(4):863-867. DOI: 10.1007/s13555-020-00396-6. Epub 2020 May 22

[123] Rajan A, Kim C, Heery CR, Guha U, Gulley JL. Nivolumab, anti-programmed death-1 (PD-1) monoclonal antibody immunotherapy: Role in advanced cancers. *Human Vaccines & Immunotherapeutics*. 2016;**12**(9):2219-2231. DOI: 10.1080/21645515.2016.1175694. Epub 2016 May 2

[124] Vasiliadou I, Breik O, Baker H, Leslie I, Sim VR, Hegarty G, et al. Safety and treatment outcomes of Nivolumab for the treatment of

recurrent or metastatic head and neck squamous cell carcinoma: Retrospective multicenter cohort study. *Cancers* (Basel). 2021;**13**(6):1413. DOI: 10.3390/cancers13061413

[125] Polverini PJ, D'Silva NJ, Lei YL. Precision therapy of head and neck squamous cell carcinoma. *Journal of Dental Research*. 2018;**97**(6):614-621. DOI: 10.1177/0022034518769645. Epub 2018 Apr 12

[126] Falchook GS, Leidner R, Stankevich E, Piening B, Bifulco C, Lowy I, et al. Responses of metastatic basal cell and cutaneous squamous cell carcinomas to anti-PD1 monoclonal antibody REGN2810. *Journal for Immunotherapy of Cancer*. 2016;**4**:70. DOI: 10.1186/s40425-016-0176-3

[127] Desai J, Deva S, Lee JS, Lin CC, Yen CJ, Chao Y, et al. Phase IA/IB study of single-agent tislelizumab, an investigational anti-PD-1 antibody, in solid tumors. *Journal for Immunotherapy of Cancer*. 2020;**8**(1):e000453. DOI: 10.1136/jitc-2019-000453

[128] Yang Y, Wang Z, Fang J, Yu Q, Han B, Cang S, et al. Efficacy and safety of Sintilimab plus Pemetrexed and platinum as first-line treatment for locally advanced or metastatic nonsquamous NSCLC: A randomized, double-blind, phase 3 study (oncology program by InnovENT anti-PD-1-11). *Journal of Thoracic Oncology*. 2020;**15**(10):1636-1646. DOI: 10.1016/j.jtho.2020.07.014. Epub 2020 Aug 8

[129] Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after Chemoradiotherapy in stage III non-small-cell lung cancer. *The New England Journal of Medicine*. 2017;**377**(20):1919-1929. DOI: 10.1056/NEJMoa1709937. Epub 2017 Sep 8

[130] Ascierto PA, Del Vecchio M, Robert C, Mackiewicz A, Chiarion-Sileni V, Arance A, et al. Ipilimumab 10 mg/kg versus ipilimumab 3 mg/kg in patients with unresectable or metastatic melanoma: A randomised, double-blind, multicentre, phase 3 trial. *The Lancet Oncology*. 2017;**18**(5):611-622. DOI: 10.1016/S1470-2045(17)30231-0. Epub 2017 Mar 27

[131] Sunshine J, Taube JM. PD-1/ PD-L1 inhibitors. *Current Opinion in Pharmacology*. 2015;**23**:32-38. DOI: 10.1016/j.coph.2015.05.011. Epub 2015 Jun 2

[132] Siu LL, Even C, Mesía R, Remenar E, Daste A, Delord JP, et al. Safety and efficacy of Durvalumab with or without Tremelimumab in patients with PD-L1-low/negative recurrent or metastatic HNSCC: The phase 2 CONDOR randomized clinical trial. *JAMA Oncology*. 2019;**5**(2):195-203. DOI: 10.1001/jamaoncol.2018.4628

[133] Haddad RI, Harrington K, Tahara M, Ferris RL, Gillison M, Fayette J, et al. Nivolumab plus Ipilimumab versus EXTREME regimen as first-line treatment for recurrent/ metastatic squamous cell carcinoma of the head and neck: The final results of CheckMate 651. *Journal of Clinical Oncology*. 2023;**41**(12):2166-2180. DOI: 10.1200/JCO.22.00332. Epub 2022 Dec 6

[134] Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. *The New England Journal of Medicine*. 2013;**369**(2):122-133. DOI: 10.1056/NEJMoa1302369. Epub 2013 Jun 2. Erratum in: *N Engl J Med*. 2018 Nov 29;**379**(22):2185. DOI: 10.1056/NEJMr180040

- [135] Basudan AM. The role of immune checkpoint inhibitors in cancer therapy. *Clinics and Practice*. 2022;**13**(1):22-40. DOI: 10.3390/clinpract13010003
- [136] Pietrobon V, Marincola FM. Hypoxia and the phenomenon of immune exclusion. *Journal of Translational Medicine*. 2021;**19**(1):9. DOI: 10.1186/s12967-020-02667-4
- [137] Burtneß B. First-line nivolumab plus Ipilimumab in recurrent/metastatic head and neck cancer-what happened? *Journal of Clinical Oncology*. 2023;**41**(12):2134-2137. DOI: 10.1200/JCO.22.02349. Epub 2023 Mar 6
- [138] Ho WJ, Yarchoan M, Hopkins A, Mehra R, Grossman S, Kang H. Association between pretreatment lymphocyte count and response to PD1 inhibitors in head and neck squamous cell carcinomas. *Journal for Immunotherapy of Cancer*. 2018;**6**(1):84. DOI: 10.1186/s40425-018-0395-x
- [139] Saddawi-Konefka R, O'Farrell A, Faraji F, Clubb L, Allevato MM, Jensen SM, et al. Lymphatic-preserving treatment sequencing with immune checkpoint inhibition unleashes cDC1-dependent antitumor immunity in HNSCC. *Nature Communications*. 2022;**13**(1):4298. DOI: 10.1038/s41467-022-31941-w
- [140] Franken A, Bila M, Mechels A, Kint S, Van Dessel J, Pomella V, et al. CD4+ T cell activation distinguishes response to anti-PD-L1+anti-CTLA4 therapy from anti-PD-L1 monotherapy. *Immunity*. 2024;**57**(3):541-558.e7. DOI: 10.1016/j.immuni.2024.02.007. Epub 2024 Mar 4
- [141] Harrington KJ, Ferris RL, Gillison M, Tahara M, Argiris A, Fayette J, et al. Efficacy and safety of nivolumab plus Ipilimumab vs nivolumab alone for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck: The phase 2 CheckMate 714 randomized clinical trial. *JAMA Oncology*. 2023;**9**(6):779-789. DOI: 10.1001/jamaoncol.2023.0147
- [142] Ferris RL, Haddad R, Even C, Tahara M, Dvorkin M, Ciuleanu TE, et al. Durvalumab with or without tremelimumab in patients with recurrent or metastatic head and neck squamous cell carcinoma: EAGLE, a randomized, open-label phase III study. *Annals of Oncology*. 2020;**31**(7):942-950. DOI: 10.1016/j.annonc.2020.04.001. Epub 2020 Apr 12
- [143] Psyrri A, Fayette J, Harrington K, Gillison M, Ahn MJ, Takahashi S, et al. Durvalumab with or without tremelimumab versus the EXTREME regimen as first-line treatment for recurrent or metastatic squamous cell carcinoma of the head and neck: KESTREL, a randomized, open-label, phase III study. *Annals of Oncology*. 2023;**34**(3):262-274. DOI: 10.1016/j.annonc.2022.12.008. Epub 2022 Dec 16
- [144] Cruz E, Kayser V. Monoclonal antibody therapy of solid tumors: Clinical limitations and novel strategies to enhance treatment efficacy. *Biologics*. 2019;**13**:33-51. DOI: 10.2147/BTT.S166310
- [145] Qiao M, Jiang T, Ren S, Zhou C. Combination strategies on the basis of immune checkpoint inhibitors in non-small-cell lung cancer: Where do we stand? *Clinical Lung Cancer*. 2018;**19**(1):1-11. DOI: 10.1016/j.clcc.2017.06.005. Epub 2017 Jun 23
- [146] Garassino MC, Torri V, Colombo MP, Sica A. Choosing the best chemotherapy agent to boost immune checkpoint inhibition activity. *Cancer*

Research. 2018;**78**(20):5729-5730.
DOI: 10.1158/0008-5472.CAN-18-2245

[147] Lynch TJ, Bondarenko I, Luft A, Serwatowski P, Barlesi F, Chacko R, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line treatment in stage IIIB/IV non-small-cell lung cancer: Results from a randomized, double-blind, multicenter phase II study. *Journal of Clinical Oncology*. 2012;**30**(17):2046-2054. DOI: 10.1200/JCO.2011.38.4032. Epub 2012 Apr 30. Erratum in: *J Clin Oncol*. 2012 Oct 10;**30**(29):3654

[148] Suzuki R, Hamada K, Ohkuma R, Homma M, Tsurui T, Iriguchi N, et al. Case report: Combined pembrolizumab, 5-fluorouracil, and cisplatin therapy were remarkably effective in p16-positive squamous cell carcinoma of unknown primary. *Frontiers in Oncology*. 2023;**13**:1231986. DOI: 10.3389/fonc.2023.1231986

[149] Leduc C, Adam J, Louvet E, Sourisseau T, Dorvault N, Bernard M, et al. TPF induction chemotherapy increases PD-L1 expression in tumour cells and immune cells in head and neck squamous cell carcinoma. *ESMO Open*. 2018;**3**(1):e000257. DOI: 10.1136/esmoopen-2017-000257

[150] Chakraborty S, Ye J, Wang H, Sun M, Zhang Y, Sang X, et al. Application of toll-like receptors (TLRs) and their agonists in cancer vaccines and immunotherapy. *Frontiers in Immunology*. 2023;**14**:1227833. DOI: 10.3389/fimmu.2023.1227833

[151] Hui R, Liu X, Fan Z, Ji H, Wei D, Ren G. The efficacy of cetuximab plus PD-1 inhibitors as salvage therapy in PD-1 refractory patients with recurrent or metastatic head and neck squamous cell carcinoma. *Journal of Cancer*.

2024;**15**(6):1668-1674. DOI: 10.7150/jca.92689

[152] Kang SH, Keam B, Ahn YO, Park HR, Kim M, Kim TM, et al. Inhibition of MEK with trametinib enhances the efficacy of anti-PD-L1 inhibitor by regulating anti-tumor immunity in head and neck squamous cell carcinoma. *Oncoimmunology*. 2018;**8**(1):e1515057. DOI: 10.1080/2162402X.2018.1515057

[153] Trommer M, Yeo SY, Persigehl T, Bunck A, Grüll H, Schlaak M, et al. Abscopal effects in radio-immunotherapy-response analysis of metastatic cancer patients with progressive disease under anti-PD-1 immune checkpoint inhibition. *Frontiers in Pharmacology*. 2019;**10**:511. DOI: 10.3389/fphar.2019.00511. Erratum in: *Front Pharmacol*. 2020 Jan 31; **10**:1615. DOI: 10.3389/fphar.2019.00511

[154] Ko EC, Formenti SC. Radiation therapy to enhance tumor immunotherapy: A novel application for an established modality. *International Journal of Radiation Biology*. 2019;**95**(7):936-939. DOI: 10.1080/09553002.2019.1623429. Epub 2019 Jun 13

[155] Guo S, Yao Y, Tang Y, Xin Z, Wu D, Ni C, et al. Radiation-induced tumor immune microenvironments and potential targets for combination therapy. *Signal Transduction and Targeted Therapy*. 2023;**8**(1):205. DOI: 10.1038/s41392-023-01462-z

[156] Zappasodi R, Merghoub T, Wolchok JD. Emerging concepts for immune checkpoint blockade-based combination therapies. *Cancer Cell*. 2018;**34**(4):690. DOI: 10.1016/j.ccell.2018.09.008. Erratum for:

Cancer Cell. 2018 Apr 9;33(4):581-598.
DOI: 10.1016/j.ccell.2018.03.005

[157] Meci A, Goyal N, Slonimsky G.
Mechanisms of resistance and
therapeutic perspectives in
immunotherapy for advanced head
and neck cancers. *Cancers (Basel)*.
2024;**16**(4):703. DOI: 10.3390/
cancers16040703

[158] Zhao Y, Liu L, Weng L.
Comparisons of underlying
mechanisms, clinical efficacy and safety
between anti-PD-1 and anti-PD-L1
immunotherapy: The state-of-the-art
review and future perspectives. *Frontiers
in Pharmacology*. 2021;**12**:714483.
DOI: 10.3389/fphar.2021.714483

Role of Crosstalk between Microbiota and Immune System in Cancer Immunotherapy

Giuliana Ciappina, Patrizia Carroccio, Francesco Bignoli, Mariacarmela Santaripa, Giordana Di Mauro and Massimiliano Berretta

Abstract

The microbiota is a complex ecosystem composed of bacteria, viruses, fungi, and protists that colonize gut, skin, oral cavity, and vagina. Microbiota plays a crucial role in maintaining health by regulating the immune system and producing metabolites such as short-chain fatty acids (SCFAs), which contribute to the modulation of immune response. Its interaction with the immune system occurs through metabolites and the involvement of immune cells such as regulatory and dendritic T-cells, influencing inflammatory processes and the anticancer response. Several studies demonstrate that the microbiota also affects the efficacy of immunotherapy, particularly immune checkpoint inhibitors (ICIs) used in cancer treatment. Modulating the microbiota represents a promising therapeutic strategy to improve the response to immunotherapy. Methods such as the use of probiotics, prebiotics, antibiotics, and fecal microbiota transplantation (FMT) have been studied to restore a favorable microbiota, enhancing treatment efficacy.

Keywords: gut microbiota, immune system, cancer, immunotherapy, chronic inflammation

1. Introduction

The gut microbiota represents a complex ecosystem consisting of a wide range of microorganisms, including bacteria, viruses, fungi and protists, that colonize the gastrointestinal tract. Bacteria represent the principal component of the microbiota, with Firmicutes, Bacteroidetes, and Actinobacteria being the most prominent phyla [1]. This system of microorganisms plays a crucial role in maintaining the balance of human health, contributing to protection against pathogens through the production of metabolites, including short-chain fatty acids (SCFAs), such as acetate, propionate and butyrate that result from the fermentation of dietary fiber by gut bacteria [2, 3].

One of the most relevant functions of microbiota is its ability to regulate the immune system by inhibiting pro-inflammatory immune cells and stimulating the differentiation and function of immunosuppressive cells. Due to these fundamental functions, the healthy state of microbiota, known as eubiosis, is essential for maintaining homeostasis. Alterations in the composition and diversity of microbiota, known as dysbiosis, have been associated with several diseases, particularly chronic inflammatory conditions such as inflammatory bowel diseases (IBDs), but also with mechanisms of carcinogenesis of colorectal cancer (CRC) [3]. The composition of the gut microbiota is shaped by numerous factors, including diet, environmental conditions, individual's gender, geographical location, and the use of specific drugs (**Figure 1**). Aging can alter the balance and diversity of the microbiota. From the earliest moments of life, microbial colonization of the gut is influenced by multiple elements, such as the mode of delivery (vaginal or cesarean), breastfeeding, and maternal diet [4]. These factors play a crucial role in the development of the infant's immune system and metabolism, laying the foundation for future health [5]. Over the years, the abundance of beneficial bacteria tends to decrease, while potentially pathogenic species may increase. One of the main element that modulates the composition of the microbiota is diet. Western diets high in saturated fats, refined sugars, and ultra-processed elements and low in fiber cause an increase in pro-inflammatory bacteria in the gut [6]. Conversely, fiber-rich foods such as fruits and vegetables, whole grains, and probiotic-rich foods promote a microbiota rich in beneficial bacterial species that exert an anti-inflammatory effect [7]. When discussing diet, it is essential to consider not only the types and quality of nutrients consumed but also mealtimes and overall eating habits. These factors significantly influence gut microbiota. For example, irregular eating patterns, prolonged fasting, or frequent snacking can disrupt the circadian rhythm of the microbiota, leading to imbalances in microbial composition. In contrast, consistent meal timing and a balanced dietary routine support microbial diversity and stability. Therefore, both what we eat and how we eat play a crucial role

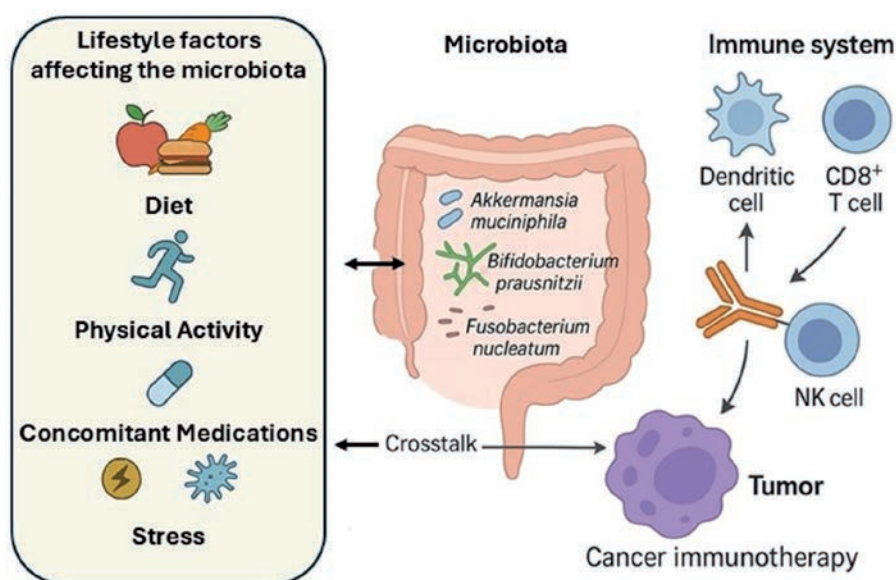


Figure 1.
The interaction between lifestyle factors, microbiota, and immune system.

in shaping the gut microbial ecosystem and, consequently, in maintaining overall health [8]. The benefits of a healthy diet in modulating gut microbiota are amplified when combined with other lifestyle habits: not smoking, maintaining a healthy weight, drinking alcohol in moderation, and engaging in regular physical activity [5]. In fact, regular physical activity has also been shown to positively influence the composition and diversity of the gut microbiota. Specifically, it enhances microbial biodiversity by increasing the abundance of beneficial bacterial species involved in maintaining gut homeostasis [9]. Moreover, physically active individuals tend to have a more resilient and stable microbiota, better equipped to adapt to stressors such as dietary changes or illness [10]. Thus, incorporating regular exercise into one's lifestyle not only benefits cardiovascular and muscular health but also supports a balanced and diverse gut microbial ecosystem. Moreover, the aging process involves a progressive loss of muscle mass and strength, a phenomenon known as sarcopenia, which typically begins around the age of 40. However, engaging in regular physical activity can significantly delay muscle loss and the accumulation of body fat, thereby helping to prevent sarcopenia. This condition is associated with low-grade systemic inflammation and metabolic dysfunction, both of which can disrupt the balance of gut microbiota, promoting dysbiosis and negatively affecting overall health. Furthermore, the increased body fat often accompanying sarcopenia has also been linked to alterations in gut microbiota composition [11]. Other factors that can induce dysbiosis include chronic comorbidities such as obesity and type 2 diabetes. These conditions are often characterized by systemic low-grade inflammation, insulin resistance, and metabolic dysfunction, all of which can negatively affect the gut microbiota [12]. In individuals with obesity, for example, there is often a reduction in microbial diversity and an imbalance between beneficial and pathogenic bacterial species [13]. This altered microbial composition may further exacerbate inflammation and metabolic disturbances, creating a vicious cycle. Similarly, in type 2 diabetes, dysbiosis has been linked to impaired glucose metabolism and increased intestinal permeability, which can allow endotoxins to enter the bloodstream and trigger immune responses [14]. These findings highlight the bidirectional relationship between chronic diseases and the gut microbiota, where each can influence and worsen the other. Another critical factor is drug intake, which can significantly alter the composition of the microbiota. Antibiotics can drastically alter the microbiota, reducing its diversity and promoting the growth of resistant pathogen strains [15]. Other drugs can also significantly alter the composition of the gut microbiota, like proton pump inhibitors (PPIs), metformin, and statins. PPIs, commonly used to treat acid-related disorders, reduce gastric acid secretion, leading to an increased gastric pH. This change can allow the survival and proliferation of normally suppressed bacteria, resulting in an altered microbial composition in the upper gastrointestinal tract and contributing to overall dysbiosis. Long-term use of PPIs has been associated with reduced microbial diversity and an increased risk of infections such as *Clostridioides difficile* [16]. Given its systemic relevance, and particularly its capacity to interact with the immune system, the gut microbiota, especially dysbiotic states, constitutes an emerging factor in numerous medical contexts, with particular emphasis on oncology.

2. Relationship between microbiota and the immune system

The interaction between the gut microbiota and the immune system has been studied for several years; it has been observed that the development and proper

functioning of the immune system largely depend on the balance of the gut microbiota [17]. Studies in microbiota-free (germ-free) animal models have shown that the absence of microbiota impairs the development and function of the immune system, underscoring the importance of the microbiota in immune maturation. Specifically, the absence of microbiota impairs the formation of gut-associated lymphatic tissue (GALT) and reduces the production of immunoglobulin A (IgA), which is essential for mucosal protection. Gut bacteria produce metabolites such as SCFAs, including butyrate, acetate, and propionate, from the fermentation of dietary fiber. These metabolites have significant immunomodulatory effects, particularly inhibiting the production of pro-inflammatory cytokines such as IL-6 and TNF- α , contributing to a less inflammatory immune environment. In addition, SCFAs influence the differentiation of regulatory T cells (Treg) and helper T cells (Th17), modulating the immune response and maintaining intestinal homeostasis. The gut microbiota also modulates the function of dendritic cells (DCs), which are critical for the activation of immune responses, influencing the maturation of DCs and their ability to present antigens. Influence is also and especially exerted on the intestinal barrier: the microbiota contributes to the maintenance of the integrity of the intestinal barrier, preventing the translocation of pathogens and pro-inflammatory molecules into the systemic circulation. Through the production of metabolites and interaction with the mucosal immune system, the microbiota can modulate the immune response at the systemic level as well, influencing the onset of autoimmune and inflammatory diseases [17, 18].

3. Involvement of the microbiota in carcinogenesis

The alteration of the composition of the microbiota is involved in the establishment of chronic inflammatory state, and it has been associated with an increased risk of developing various neoplasms. The microbiota can influence gene expression through the production of bioactive metabolites. Pathogenic bacteria, such as *Escherichia coli*, can produce “genotoxins” like Colibactin, causing DNA damage [19]. The oral Gram-negative bacterium *Fusobacterium nucleatum* is among the most extensively researched and referenced microbial species in multiple systemic diseases, with special focus on its role as an oncogenic bacterium. Multiple studies have elucidated the dualistic role of *F. nucleatum* as both a commensal microorganism and an opportunistic pathogen. Recent evidence has suggested that significant modulation of *F. nucleatum* virulence is likely driven by perturbations in the oral microbial community [20]. Several data have demonstrated that *F. nucleatum* facilitates the adenoma-to-carcinoma transition by inducing nuclear translocation of β -catenin and upregulating the expression of the regenerative gene (REG) I α , which encodes proteins that exert proliferative and anti-apoptotic effects on inflammatory and neoplastic lesions within the gastrointestinal tract, in particular in CRC. Moreover, by negatively modulating the anti-tumor immune response, *F. nucleatum* can activate oncogenic pathways such as Wnt/ β -catenin and NF- κ B [21]. However, *F. nucleatum* is not only involved in CRC but has also been found in esophageal, gastric, pancreatic, and gynecological cancers [22]. In the same way, Adherent-Invasive *Escherichia Coli* (AIEC) can contribute to carcinogenesis through interaction with intestinal epithelial cells and the production of Reactive Oxygen Species (ROS) and Nitric Oxide (NO), exacerbating local chronic inflammation that can lead to the development of CRC [23]. Recent evidences have also suggested the involvement of the microbiota in the context of lung cancer: a true pulmonary microbiome exists also in the respiratory tract. This environment can

be altered with a predominance of pro-inflammatory bacteria such as *Streptococcus*, *Prevotella*, or *Veillonella*, capable of stimulating the production of cytokines such as IL-6 and IL-17 and promoting tumor growth, angiogenesis, and metastasis. Pro-inflammatory bacteria may also interact with pulmonary epithelial cells by activating receptors such as Toll-like receptors (TLRs) and triggering oncogenic pathways [24]. The role of the gut microbiota has not only been described in the mechanisms of carcinogenesis but is also increasingly emerging as a prognostic and predictive factor. Several studies, in fact, have demonstrated that increased abundance of *F. nucleatum* in CRC tissues has been associated with reduced patient survival [25]. Moreover, it appears that the gut microbiota may influence the response to conventional anticancer therapies through the production of specific metabolites. For example, in a state of eubiosis, certain microbial-derived SCFAs, such as butyrate and propionate, have been shown to enhance the efficacy of chemotherapeutic agents and immune checkpoint inhibitors (ICIs). Conversely, dysbiosis and the loss of beneficial microbial functions may impair drug metabolism or promote resistance, ultimately reducing treatment effectiveness [26].

4. Influence of microbiota on immunotherapy

Immunotherapy revolutionized cancer treatment, particularly with ICIs such as anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies. However, not all patients respond effectively to such therapies and numerous studies have shown that the microbiota plays a key role in these differences in response [18]. In particular, it has been observed that patients who respond positively to ICIs have a microbiota characterized by specific bacteria capable of stimulating anti-tumor immune responses [27]. Research efforts have led to the description of several Gut OncoMicrobiome Signatures (GOMS) that could become prognostic and predictive markers across several cancer types. Preliminary evidence indicates that cancer patients who are more likely to respond to immunotherapy tend to harbor a gut microbiota resembling the prototypical healthy GOMS [28]. Among the most studied bacteria in this field, *Akkermansia muciniphila* has been shown to improve clinical outcomes and prolong progression-free survival in patients with advanced non-small-cell lung cancer (NSCLC) treated with immunotherapy [29]. Similarly, in patients with advanced melanoma, *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* were correlated with a better response to anti-PD-1 [30]. Studies in mouse models show that the transfer of a mixture of IFN- γ -producing bacteria enhances the efficacy of anti-PD-1 by promoting the infiltration of CD8⁺ T-cells into the tumor [31]. Conversely, a state of dysbiosis may compromise the intestinal barrier, promoting chronic inflammation and reducing the efficacy of immunotherapy [27, 32]. As previously mentioned, drugs such as antibiotics and PPIs can significantly affect the composition of the intestinal microbiota and, consequently, response rates and tolerability to ICIs [33]. In a recent study published by Elkrief et al., the role of concomitant antibiotic use was evaluated in patients with advanced lung cancer undergoing chemoimmunotherapy or monotherapy with ICIs. A total of 2028 patients were included in the study, and antibiotic exposure was associated with a lower objective response rate (ORR: 27% vs. 40%, $p = 0.001$), shorter progression-free survival (PFS: 3.9 months vs. 5.9 months; hazard ratio [HR] 1.35, 95% CI: 1.1–1.6, $p = 0.0012$), and reduced overall survival (OS: 10 months vs. 15 months; HR 1.50, 95% CI: 1.2–1.8, $p = 0.00014$) [34]. Regarding PPIs, a meta-analysis conducted by Lopes et al. demonstrated that

the concomitant use of this widely prescribed drug class can significantly impair the efficacy of immunotherapy, regardless of cancer type [33]. In addition to their impact on treatment efficacy, recent studies have suggested that PPIs may also influence immune-related toxicity. For example, in a retrospective study by Lasagna et al., chronic PPIs use was associated with a significantly increased incidence of gastrointestinal immune-related adverse events (GI irAEs) in patients treated with ICIs. Among 363 patients, 92% of those exposed to PPIs developed GI irAEs, compared to only 8% of unexposed patients. (hazard ratio [HR] 13.22, 95% confidence interval [CI] 3.11–56.10, $p < 0.000$) [35]. These results highlight the need for prospective studies to better elucidate the causal relationship between dysbiosis and immunotherapy outcomes. A deeper understanding of these interactions is essential to optimize treatment strategies, minimize adverse effects, and ultimately improve the clinical benefit of immune checkpoint inhibitors across different cancer types.

5. Modulation of the microbiota

Considering the influence of the microbiota on therapeutic outcomes, its modulation could represent an opportunity to develop new personalized therapeutic approaches in cancer treatment. Strategies to modify the microbiota include the use of probiotics, prebiotics, antibiotics, and fecal microbiota transplantation (FMT) [36]. A central role is also played by lifestyle changes, particularly diet: the Mediterranean diet is one of the most effective for promoting intestinal microbiota balance, due to its high intake of fiber, polyphenols, unsaturated fatty acids, and a low presence of ultra-processed foods. It stimulates the growth of beneficial bacteria like *Bifidobacterium* and *Lactobacillus*, improving intestinal health, strengthening the epithelial barrier, and reducing chronic inflammatory states [37]. Probiotics, live microorganisms that colonize the intestine, and prebiotics, nondigestible substrates that promote the growth of beneficial bacteria, are additional tools to improve microbiota composition [38]. In a phase 1 trial by Ebrahimi et al., the integration of probiotics like *Bifidobacterium* with ICIs for metastatic renal cell carcinoma has demonstrated improvement in ORR [39]. One of the most extensively studied approaches in this context is FMT. FMT involves the transfer of microbes from healthy donors to patients with dysbiosis *via* endoscopy, enema, or oral capsules [40]. The effect of FMT on the response to ICIs was initially investigated in preclinical mouse models, where it was shown to have the potential to reverse resistance to ICIs [41]. In a phase 1 study by Routy et al., the safety and efficacy of FMT in combination with nivolumab or pembrolizumab were evaluated in 20 treatment-naïve patients with advanced melanoma, demonstrating that the combination with single-agent anti-PD-1 therapy was well tolerated. Furthermore, the observed ORR was 65%, significantly higher than the 45% reported in historical controls [42]. This study marked a pivotal advancement in the FMT/ICI field, demonstrating that FMT from healthy donors can enhance the antitumor efficacy of immune checkpoint inhibitors and significantly improve the feasibility of FMT as a scalable therapeutic strategy. Other studies are evaluating the use of microbiome ecosystem therapies (MET) that combine specific bacterial strains with anti-PD-1/PD-L1 therapies to optimize immune response and improve clinical outcomes, increasing tolerability compared to FMT [43]. These data suggest that manipulating the intestinal microbiota could be a promising strategy to improve responses to oncological therapies, address treatment resistance, and optimize the overall survival of patients. The combination of microbiota and

Study (NCT)	Objective	Cancer type	Microbiota-related intervention	Phase
NCT03341143	Evaluate whether FMT improves pembrolizumab response in PD-1-refractory melanoma	Advanced melanoma	Fecal microbiota transplant + pembrolizumab	Phase 2
NCT04729322	Evaluate FMT + rechallenge with pembrolizumab/nivolumab in non-responding dMMR CRC	Metastatic colorectal cancer (dMMR)	FMT capsules + anti-PD-1	Phase 2
NCT04975217	Assess safety/tolerability of FMT in resectable pancreatic cancer patients	Resectable pancreatic cancer	Colonoscopy-based and capsule FMT	Early Phase 1
NCT05865730	Test Oncobax®-AK (A. muciniphila) to enhance ICI efficacy in Akkermansia-deficient NSCLC/RCC	NSCLC, metastatic renal cell carcinoma	Oral <i>Akkermansia muciniphila</i> (capsules)	Phase 1/2
NCT06349590	Evaluate whether a fiber-rich, low-fat diet reduces recurrence post-surgery in CRC	Resectable primary colorectal cancer	3-week standardized high-fiber, low-fat diet	Phase 1/2
NCT03819296	Study FMT from healthy donors + immunotherapy in various advanced solid tumors	Multiple (melanoma, GU, lung)	FMT capsules from healthy donors + ICI	Phase 1/2
NCT04163289	Evaluate FMT capsules in metastatic RCC treated with nivolumab/ipilimumab to reduce occurrence of immune-related toxicities	Metastatic renal cell carcinoma	FMT from donor + anti-PD-1/anti-CTLA-4	Phase 1
NCT04758507	Test FMT capsules from ICI-responders donors in combination with ICI in advanced RCC	Advanced renal cell carcinoma	FMT capsules + immunotherapy	Phase 1/2

Table 1.
 Ongoing clinical trials on microbiota and cancer.

immunological therapies could offer new approaches for cancer treatment, with potentially more durable and less toxic results. Several clinical trials are currently evaluating the efficacy of microbiota modulation in improving responses to cancer treatments, particularly immunotherapy (**Table 1**).

6. Conclusion

Scientific evidences show that the gut microbiota plays a key role in regulating health and immune response. Its composition directly influences intestinal homeostasis, protection against pathogens, and modulation of inflammatory responses. Alterations in the microbiota may contribute to the development of chronic and oncological diseases, influencing disease progression and response to therapies. The microbiota is a key element to determinate the efficacy of immunotherapy, particularly ICIs. The presence of specific beneficial bacteria was associated with an increased likelihood of treatment success, whereas a microbial imbalance may hinder the positive effects. So, modulation of the microbiota could be a promising strategy to optimize therapeutic approaches. The mediterranean diet, the use of probiotics,

and FMT represent innovative tools to improve the immune response and increase the efficacy of cancer therapies. Therefore, future research should focus on a deeper understanding of the mechanisms by which the microbiota interacts with the immune system, with the aim of developing personalized strategies for the prevention and treatment of cancer and immune diseases.

Conflict of interest

The authors declare no conflict of interest.

Author details

Giuliana Ciappina^{1†}, Patrizia Carroccio^{2†}, Francesco Bignoli²,
Mariacarmela Santarpia³, Giordana Di Mauro² and Massimiliano Berretta^{4,5*}

1 Department of Medical Sciences, Section of Experimental Medicine, University of Ferrara, Ferrara, Italy

2 School of Specialization in Medical Oncology, Department of Human Pathology “G. Barresi”, University of Messina, Messina, Italy

3 Medical Oncology Unit, Department of Human Pathology of Adulthood and Childhood “G. Barresi”, University of Messina, Messina, Italy

4 Division of Medical Oncology, Policlinico “G. Martino” Hospital, University of Messina, Messina, Italy

5 Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy

*Address all correspondence to: mberretta@unime.it

†These authors have contributed equally to this work and share first authorship.

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Schmitt M, Greten FR. The inflammatory pathogenesis of colorectal cancer. *Nature Reviews. Immunology*. 2021;**21**(10):653-667
- [2] Réthi-Nagy Z, Juhász S. Microbiome's universe: Impact on health, disease and cancer treatment. *Journal of Biotechnology*. 2024;**392**:161-179
- [3] Wang J, Zhu N, Su X, Gao Y, Yang R. Gut-microbiota-derived metabolites maintain gut and systemic immune homeostasis. *Cells*. 2023;**12**(5):793
- [4] Schoultz I, Claesson MJ, Dominguez-Bello MG, Fåk Hållenius F, Konturek P, Korpela K, et al. Gut microbiota development across the lifespan: Disease links and health-promoting interventions. *Journal of Internal Medicine*. 2025;**297**(6):560-583
- [5] Conlon MA, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. *Nutrients*. 2014;**7**(1):17-44
- [6] El-Sayed A, Aleya L, Kamel M. Microbiota's role in health and diseases. *Environmental Science and Pollution Research International*. 2021;**28**(28):36967-36983
- [7] Aziz T, Hussain N, Hameed Z, Lin L. Elucidating the role of diet in maintaining gut health to reduce the risk of obesity, cardiovascular and other age-related inflammatory diseases. *Gut Microbes*. 2024;**16**(1):2297864.liu
- [8] Zhang P. Influence of foods and nutrition on the gut microbiome and implications for intestinal health. *International Journal of Molecular Sciences*. 2022;**23**(17):9588
- [9] Monda V, Villano I, Messina A, Valenzano A, Esposito T, Moscatelli F, et al. Exercise modifies the gut microbiota with positive health effects. *Oxidative Medicine and Cellular Longevity*. 2017;**2017**:3831972
- [10] Wegierska AE, Charitos IA, Topi S, Potenza MA, Montagnani M, Santacroce L. The connection between physical exercise and gut microbiota: Implications for competitive sports athletes. *Sports Medicine*. 2022;**52**(10):2355-2369
- [11] Grosicki GJ, Fielding RA, Lustgarten MS. Gut microbiota contribute to age-related changes in skeletal muscle size, composition, and function. *Calcified Tissue International*. 2018;**102**(4):433-442
- [12] Peña-Durán E, García-Galindo JJ, López-Murillo LD, Huerta-Huerta A, Balleza-Alejandri LR, Beltrán-Ramírez A, et al. Microbiota and inflammatory markers: A review. *International Journal of Molecular Sciences*. 2025;**26**(4):1773
- [13] Liu BN, Liu XT, Liang ZH, Wang JH. Gut microbiota in obesity. *World Journal of Gastroenterology*. 2021;**27**(25):3837-3850
- [14] Garcia-Gutierrez E, O'Mahony AK, Dos Santos RS, Marroquí L, Cotter PD. Gut microbial metabolic signatures in diabetes mellitus. *Gut Microbes*. 2024;**16**(1):2401654
- [15] Huang C, Feng S, Huo F, Liu H. Effects of four antibiotics on the diversity of the intestinal microbiota. *Microbiology Spectrum*. 2022;**10**(2):e0190421

- [16] Lopes S, Pabst L, Dory A, Klotz M, Gourieux B, Michel B, et al. Do PPIs alter response to checkpoint inhibitors? *Frontiers in Immunology*. 2023;**14**:1070076
- [17] Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Research*. 2020;**30**(6):492-506
- [18] Li Z, Xiong W, Liang Z, Wang J, Zeng Z, Kołat D, et al. Critical role of the gut microbiota in immune responses and cancer immunotherapy. *Journal of Hematology & Oncology*. 2024;**17**(1):33
- [19] Wang Y, Fu K. Genotoxins: The mechanistic links between *Escherichia coli* and colorectal cancer. *Cancers (Basel)*. 2023;**15**(4):1152
- [20] Scano A, Fais S, Ciappina G, Genovese M, Granata B, Montopoli M, et al. Oxidative stress by H₂O₂ as a potential inductor in *Fusobacterium nucleatum*. *Antioxidants (Basel)*. 2025;**14**(3):323
- [21] Mondal T, Chattopadhyay D, Saha Mondal P, Das S, Mondal A, Das A, et al. *Fusobacterium nucleatum* modulates the Wnt/ β -catenin pathway in colorectal cancer. *International Journal of Biological Macromolecules*. 2025;**299**:140196
- [22] Alon-Maimon T, Mandelboim O, Bachrach G. *Fusobacterium nucleatum* and cancer. *Periodontology 2000*. 2000;**89**(1):166-180
- [23] Qu R, Zhang Y, Ma Y, Zhou X, Sun L, Jiang C, et al. Role of the gut microbiota and its metabolites in colorectal cancer. *Advanced Science*. 2023;**10**(23):e2205563
- [24] Zhao Y, Liu Y, Li S, Peng Z, Liu X, Chen J, et al. Role of lung and gut microbiota on lung cancer pathogenesis. *Journal of Cancer Research and Clinical Oncology*. 2021;**147**(8):2177-2186
- [25] Martin-Gallausiaux C, Salesse L, Garcia-Weber D, Marinelli L, Beguet-Crespel F, Brochard V, et al. *Fusobacterium nucleatum* promotes inflammatory responses via ADP-heptose. *Gut Microbes*. 2024;**16**:2295384
- [26] Al-Qadami GH, Secombe KR, Subramaniam CB, Wardill HR, Bowen JM. Short-chain fatty acids: Impact on cancer treatment. *Microorganisms*. 2022;**10**(10):2048
- [27] Lu Y, Yuan X, Wang M, et al. Gut microbiota influence immunotherapy responses. *Journal of Hematology & Oncology*. 2022;**15**:47
- [28] Thomas AM, Fidelle M, Routy B, Kroemer G, Wargo JA, Segata N, et al. Gut OncoMicrobiome signatures as biomarkers for immunotherapy. *Nature Reviews. Clinical Oncology*. 2023;**20**(9):583-603
- [29] Derosa L, Routy B, Thomas AM, Iebba V, Zalcman G, Friard S, et al. Intestinal *Akkermansia muciniphila* predicts response to PD-1 blockade. *Nature Medicine*. 2022;**28**(2):315-324
- [30] Matson V, Fessler J, Bao R, Chongsuwat T, Zha Y, Alegre ML, et al. Commensal microbiome and anti-PD-1 efficacy in melanoma. *Science*. 2018;**359**(6371):104-108
- [31] Kang X, Lau HC, Yu J. Modulating gut microbiome in cancer immunotherapy. *Cell Reports Medicine*. 2024;**5**(4):101478
- [32] Muscolino P, Granata B, Omero F, De Pasquale C, Campana S, Calabrò A, et al. Gut microbiota in immunotherapy of HCC patients. *Frontiers in Oncology*. 2023;**13**:1247614

- [33] Lange K, Buerger M, Stallmach A, Bruns T. Effects of antibiotics on gut microbiota. *Digestive Diseases*. 2016;**34**(3):260-268
- [34] Elkrief A, Méndez-Salazar EO, Maillou J, Vanderbilt CM, Gogia P, Desilets A, et al. Antibiotics and outcomes in lung cancer patients. *npj Precision Oncology*. 2024;**8**(1):143
- [35] Lasagna A, Mascaro F, Figini S, Basile S, Gambini G, Klersy C, et al. PPIs and GI immune-related events in immunotherapy. *Cancer Medicine*. 2023;**12**(19):19530-19536
- [36] Radoš L, Golčić M, Mikolašević I. Gut microbiota and response to immunotherapy in cancer. *Biomedicine*. 2025;**13**(1):96
- [37] Perrone P, D'Angelo S. Mediterranean diet and gut microbiota. *Nutrients*. 2025;**17**(6):948
- [38] Wang X, Zhang P, Zhang X. Probiotics regulate gut microbiota and improve immunity. *Molecules*. 2021;**26**(19):6076
- [39] Ebrahimi H, Dizman N, Meza L, et al. Cabozantinib and nivolumab with/without bacteria in RCC. *Nature Medicine*. 2024;**30**:2576-2585
- [40] Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, et al. Fecal microbiota transplant overcomes anti-PD-1 resistance. *Science*. 2021;**371**(6529):595-602
- [41] Daillère R, Routy B, Goubet AG, Cogdill A, Ferrere G, Alves-Costa Silva C, et al. Immunostimulatory commensals and checkpoint inhibitors. *Oncoimmunology*. 2020;**9**(1):1794423
- [42] Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science*. 2018;**359**(6371):97-103. DOI: 10.1126/science.aan4236
- [43] Andrews MC, Duong CPM, Gopalakrishnan V, Iebba V, Chen WS, Derosa L, et al. Gut microbiota signatures are associated with toxicity to combined CTLA-4 and PD-1 blockade. *Nature Medicine*. 2021;**27**(2):143-153. DOI: 10.1038/s41591-020-01188-4

Section 3

Immunotherapy in Action: Strategies for Different Cancers

Immunology and Gliomas: Present and Future Opportunities in Diagnostics and Therapy

Anna Bilotta, Deborah Dardano and Giuseppe Donato

Abstract

According to the Central Brain Tumor Registry of the United States (CBTRUS), malignant primary brain tumors cause more than 15,000 deaths per year. The annual incidence is approximately 7 per 100,000 individuals and increases with age. The five-year survival rate is approximately 36%. Gliomas are the most prevalent and aggressive adult-type primary tumors of the central nervous system (CNS). These tumors range from lower-grade gliomas (LGGs) to the highly malignant glioblastoma multiforme (GBM), defined as isocitrate dehydrogenase (IDH)-wild-type diffuse glioma. Anatomic magnetic resonance imaging (MRI) and fluid-attenuated inversion recovery (FLAIR) are the gold standards for diagnosing and following up on GBM, providing macrostructural anatomical information. Despite many therapeutic strategies involving maximal surgical resection, radiotherapy, and alkylating chemotherapy, the prognosis remains poor. Gliomas are very low-immunogenic tumors, called “immune-cold,” located in a T-cell and other immune cell-poor CNS compartment, due to poor infiltration of cytotoxic T lymphocytes (CTLs) and the dominance of immunosuppressive myeloid populations. The extracellular matrix (ECM) and the blood-brain barrier (BBB) play a relevant role in maintaining the immunosuppressive tumor microenvironment (TME). It predominantly consists of microglia and macrophages, but it also includes other myeloid cells, such as bone marrow-derived macrophages (BMDMs), neutrophils, and myeloid-derived suppressor cells (MDSCs). Immunotherapy for CNS tumors, along with trials involving immune checkpoint inhibitors (ICIs), therapeutic vaccines, adoptive cell therapy, monoclonal antibodies, oncolytic viruses, and other molecules, promises to be an exciting avenue of research; however, novel approaches are needed. Classical immunohistochemical techniques or those combined with confocal microscopy can open up new frontiers in diagnostics and immunotherapy planning.

Keywords: glioblastoma, innate and adaptive immunity, immune tumor microenvironment, immunotherapy, instant digital pathology, antibodies

1. Introduction

According to the 2021 World Health Organization (WHO) classification of Tumors of the Central Nervous System, 5th Edition (CNS5), low-grade gliomas are more common in children (pediatric type), with only a 7% rate of malignant

transformation from low-grade gliomas [1]. In contrast, adult-type gliomas are more commonly linked to high-grade gliomas and have a transformation rate of at least 50%, resulting in a higher incidence 14.5% of secondary glioblastoma multiforme (GBM) [2, 3]. Histology, immunohistochemistry (IHC), and molecular profiling are the three different approaches on which the WHO classification of CNS tumors is based. Adult-type diffuse gliomas, mainly GBM isocitrate dehydrogenase (IDH)-wild-type and astrocytoma IDH-mutant tumors, represent the most significant neoplasms in the practice of adult neuro-oncology [3, 4].

For the evaluation of brain tumors, the preferred imaging modality is magnetic resonance imaging (MRI), performed before and after the administration of a gadolinium-based contrast agent. A tumor biopsy, considering histopathological and molecular characteristics, is necessary for a diagnosis [5]. Treatment varies by tumor type, but the first-line therapy for GBM is surgery, followed by radiation and the alkylating chemotherapeutic agent temozolomide [1, 6]. Resection as extensive as safely possible is the cornerstone of treatment in most gliomas and is now also recommended early in the treatment of patients with radiological evidence of histologically low-grade tumors [1]. Despite the adoption of multimodal therapeutic strategies, the prognosis remains poor, with GBM patients facing a median survival of approximately 15 months and a two-year survival rate of 26–33% [1, 4, 6]. Some treatments can only improve progression-free survival, such as the addition of bevacizumab to standard treatment [6, 7].

Recent advances in molecular diagnostics—particularly, the classification of gliomas based on IDH mutation and short arm of chromosome 1 (1p)/long arm of chromosome 19 (19q) codeletion status—have provided better prognostic stratification and more targeted therapeutic options [4, 8]. Actually, a curative treatment is not available; however, for some patients with IDH-mutant tumors, combined modality therapy has resulted in long-term survival. Dose-intensified temozolomide or lomustine, in patients with progressive GBM, is used in cases of *MGMT* (O6-methylguanine-DNA-methyltransferase) promoter methylation, considered a strong prognostic biomarker. Only a minor role in the treatment of adult-type diffuse glioma has targeted treatments based on molecular alterations, today limited to patients with a genetic mutation that involves the substitution of a valine residue [V] with glutamic acid [E] at codon 600 (V600E) of the protein encoded by the B-Raf proto-oncogene, serine/threonine kinase (BRAF) gene [1].

Unlike other solid tumors, such as melanoma or non-small cell lung cancer (NSCLC), gliomas have shown limited responsiveness to immune checkpoint inhibitors (ICIs) and other immunotherapy regimens. Gliomas generally exhibit an immunosuppressive environment, called “immunologically cold,” with lower levels of T cells and neutrophils. By contrast, IDH-wild-type gliomas and brain metastases exhibit a more heterogeneous immune landscape that includes a significant presence of monocyte-derived macrophages, neutrophils, and adaptive immune cells, leading to more complex immune interactions [5]. This unresponsiveness can be attributed to several factors: the relative immune-privileged nature of CNS, the presence of the blood-brain barrier (BBB), the low tumor mutational burden (TMB) of gliomas, and the presence of an immunosuppressive tumor microenvironment (TME) [9].

The neoplastic cells and cells of the TME actively interact with each other. This interplay assists in increased immunosuppressive environment, with enhanced tumor aggressiveness and migratory potential [1]. The immunosuppressive environment not only limits the efficacy of immunotherapies but may also contribute to resistance to other therapies, including standard-of-care treatments [5, 10].

Targeting the TME has demonstrated potential, with immunotherapies such as checkpoint inhibitors and neoadjuvant therapies enhancing immune responses [5]. Overall, combinatorial and personalized strategies are likely necessary to overcome the intrinsic resistance of gliomas to immune attack.

This chapter explores the immune landscape of gliomas, focusing on innate and adaptive immune players, their interactions with tumor cells, current immunotherapeutic strategies, and opportunities for diagnostics and therapy. Emphasis is placed on the integration of immunology and diagnostics for the study of neoplasia, through the behavior of the TME, as well as on emerging and promising digital diagnostic approaches.

2. The glioma immune microenvironment

The TME is a dynamic and integral component of solid tumors, comprising not only malignant cells but also various supportive elements, such as stromal cells, glioma cells, fibroblasts, immune cells, neurons, astrocytes, the BBB, and components like the extracellular matrix (ECM) [10]. The TME plays an important role in tumor progression, immune evasion, and resistance to therapy in various cancers, including gliomas [5].

The glioma immune TME is a unique and complex structure characterized by restricted immune surveillance, dominance of innate immunity, and active

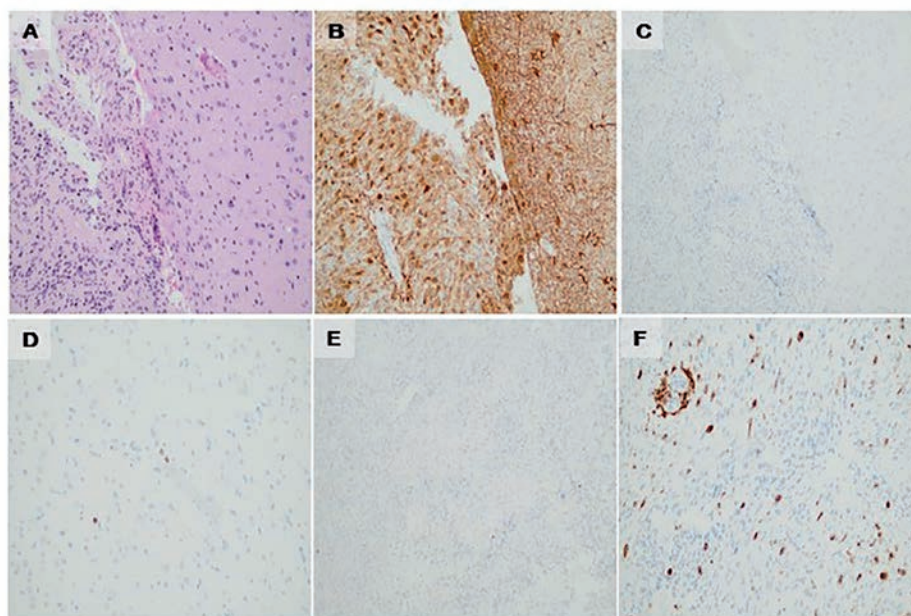


Figure 1.
 Expression of markers in a high-grade glial neoplasm by immunohistochemistry. 20x microscope acquisitions. (A) Hematoxylin and eosin stain. The glioma presence is shown on the left, near a fragment of cerebral cortex on the right with aspects of reactive gliosis. (B) Image of the same tumor sample immunolabeled with an anti-GFAP (Glial Fibrillary Acidic Protein) monoclonal antibody (Positive). (C) Immunolabeling with an anti-CD20 (cluster of differentiation 20) monoclonal antibody (Negative). (D) Immunolabeling with an anti-CD8 (cluster of differentiation 8) monoclonal antibody (some lymphocytic elements in the neoplastic tissue are positive). (E) Immunolabeling with an anti-CD4 (cluster of differentiation 4) monoclonal antibody (a single element in the neoplastic tissue is positive). (F) Immunolabeling with an anti-CD68 monoclonal antibody (several positive elements can be seen in the context of the neoplasm, including at the perivascular level).

immunosuppression. In fact, immunologically it is called a “cold tumor” due to its low lymphocytic infiltration, the absence of cancer-associated fibroblasts, and the distinct morphology of vascular endothelial cells that contribute to the BBB [10, 11]. Owing to these attributes, efficacious evidence-based treatments for GBM are exceedingly scarce, and the prognosis remains dismal [11, 12].

Microglia and bone marrow-derived macrophages (BMDMs) together constitute up to 30–50% of the glioma mass within the glioma immune microenvironment in some high-grade tumors [7]. These cells adopt tumor-supportive phenotypes that facilitate invasion, angiogenesis, and immune escape. Notably, gliomas are poorly infiltrated by effector CD8⁺ T cells and natural killer (NK) cells, while regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) are prominent. Antibodies directed against scavenger receptors, such as cluster of differentiation 68 (CD68) and cluster of differentiation 163 (CD163), the transmembrane protein 119 (TMEM119), and CD49D/ITGA4 (Integrin Subunit Alpha 4), could be used to differentiate microglia from other types of macrophages (**Figure 1**) [5, 7, 11].

The glioma microenvironment also exhibits elevated levels of immunosuppressive cytokines, such as transforming growth factor beta (TGF- β) and interleukin 10 (IL-10), and low levels of pro-inflammatory mediators like interferon gamma (IFN- γ). Additionally, tumor cells secrete chemokines that attract suppressive myeloid populations while repelling effector cells. The cumulative effect is a highly immunosuppressive milieu that promotes tumor progression and resistance to therapy [10, 12].

Spatially resolved transcriptomic studies have revealed heterogeneity in immune cell localization and function within gliomas, suggesting that localized modulation of immunity could be an effective therapeutic avenue [10, 13].

3. Extracellular matrix and physical barriers

The ECM is a dynamic set of molecules produced by the cellular components of normal and pathological tissues in both embryos and adults. ECM acts as a critical regulator in various biological processes, such as differentiation, cell proliferation, angiogenesis, and immune control [9].

Core components include collagen for strength, elastin for elasticity, fibronectin and laminin for adhesion, glycosaminoglycans for hydration, and enzymes such as matrix metalloproteinases (MMPs), which degrade ECM components to facilitate invasion and release immunomodulatory fragments, as well as lysyl oxidases (LOX) for ECM remodeling and maintenance, and other signaling molecules [5, 9].

The ECM in gliomas is not only a scaffold but also an active regulator of immunity. The brain's ECM is distinct from those of other organs due to its high concentration of glycosaminoglycans (GAGs), such as hyaluronic acid (HA), tenascin-C (TNC), and periostin (POSTN), which contribute to physical barriers that exclude immune cells from tumor nests, along with proteoglycans that modify tissue stiffness and limit T-cell mobility [5, 9]. A chondroitin sulfate proteoglycan, neural/glial antigen 2 (NG2), is implicated in GBM cell proliferation and epidermal growth factor-receptor (EGFR) signaling. A pivotal role in mediating this resistance, by promoting reactive oxygen species (ROS) scavenging and facilitating DNA damage repair, is carried out by peroxiredoxin-1 (PRDX-1). A 13- to 14-fold increase of perlecan/HSPG2 (heparin sulfate proteoglycan 2) was markedly identified. Within the HA-rich ECM, GBM cells employ cluster of differentiation 44 (CD44)-dependent microtentacles (McTNs) for adhesion and migration, creating a physical barrier that limits drug penetration and reduces efficacy [5].

Circulating ECM proteins have emerged as promising biomarkers that reflect the general immune landscape of the TME and may represent useful tools in assessing disease activity [9]. Moreover, ECM proteins can bind and sequester cytokines and chemokines, altering immune cell recruitment. Targeting the ECM through enzymatic degradation or blockade of ECM receptors may enhance immune infiltration and synergize with immunotherapies.

The CNS was classically considered an “immune-privileged site,” lacking the potential for immunosurveillance due to the presence of the BBB and the absence of a conventional lymphatic system [14]. The BBB maintains the optimal microenvironment in the CNS and is a major reason brain endothelial cells lack the expression of leukocyte adhesion molecules, preventing the entry of immune cells from the blood into the parenchyma, resulting in a paucity of immune cells in the brain microenvironment [5, 15]. However, this paradigm has recently been challenged, and it is becoming increasingly clear that the CNS interacts dynamically with the systemic immune system [11, 13].

Data indicate that leukocytes can traffic to the CNS, even with an intact BBB, and that CNS antigens can reach peripheral lymph nodes by draining cerebrospinal fluid (CSF) [10]. Brain tumors represent a particular case in which the BBB is disrupted to varying degrees, impeding the recruitment of peripheral immune cells [16]. Therefore, acquired and innate immunity may play a role in the development and growth of neoplasia [7]. Finally, the presence of specialized resident immune cells, such as microglia, shapes a distinct immunological landscape [11].

4. Myeloid cells in gliomas

Emerging evidence has highlighted the critical contribution of the immune microenvironment to tumor development and progression [11, 13].

Within the glioma TME, myeloid cells are the dominant immune population and include microglia, BMDMs, neutrophils, and MDSCs.

These cells secrete immunosuppressive cytokines, remodel the extracellular matrix, suppress T-cell activity, and adopt tumor-supportive phenotypes. For example, tumor-associated microglia and BMDMs strongly influence glioma progression [5].

4.1 Microglia

An important role in the crosstalk between tumor cells and the immune system is played by microglia. It appears in a histological section of normal brain tissue, stained with hematoxylin and eosin, in the form of ubiquitous, small, dark, elongated nuclei [7]. Two types of cells are present in the CNS: perivascular microglia and classic resident microglia [5, 11].

Tumor-associated microglia and BMDMs represent two ontogenetically distinct myeloid cell populations with similar immune regulatory features and the expression of several common antigens/markers [11]. The infiltrating BMDM population intermingles with tissue-resident microglia and becomes indistinguishable; however, microglia are defined as CD11b⁺/CD45^{low} (or CD45^{int}), whereas macrophages are defined as CD11b⁺/CD45^{high} [5, 11].

Glioma invasion is facilitated by brain-resident microglia that modulates the TME and enhances tumor proliferation through the release of cytokines, growth factors, and ECM-degrading enzymes. The proliferation of Neurofibromatosis Type

1 (NF1)-deficient astrocytes, via the secretion of hyaluronidase, is promoted by Neurofibromatosis Type 1 (NF1) heterozygous microglia [5]. Similarly, chemokine (C-C motif) ligand 5 (CCL5) promotes the growth of NF1-associated low-grade gliomas [10].

A direct link between the innate and adaptive immune systems is made by microglia. The induction of an inflammatory response by M1-like microglia/macrophages is tightly regulated through a subsequent polarity transition from a pro-inflammatory M1-polarized phenotype to an anti-inflammatory M2-like one. This results in the downregulation of immune responses, prevents tissue damage, and supports healing processes through the secretion of a series of anti-inflammatory and immune-regulating factors [11]. Glioma cell-derived factors, such as TGF- β and macrophage colony-stimulating factor (M-CSF; alternative name colony-stimulating factor 1 (CSF-1)), promote the upregulation of several M2 markers (CD163 and cluster of differentiation 204 (CD204) and thereby actively contribute to the M2-like polarization [17]. Microglia survival is critically dependent on CSF-1 receptor (CSF-1R) signaling. Macrophage survival is supported by glioma-secreted factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and IFN- γ in the presence of the CSF-1R inhibitor. Additionally, macrophage-derived insulin-like growth factor 1 (IGF-1) activates IGF-1 receptor (IGF-1R) and phosphoinositide 3-kinase (PI3K) signaling pathways in tumor cells, which promotes tumor recurrence following CSF-1R inhibition.

In preclinical GBM models, combining IGF-1R or PI3K inhibition with CSF-1R blockade markedly improves therapeutic outcomes and prolongs survival. An adenosine-enriched microenvironment in GBM is fostered by the spatial interaction between microglial cluster of differentiation 39 (CD39) and tumor-expressed cluster of differentiation 73 (CD73); it facilitates immune evasion and poor outcomes [5]. Similarly, tissue factor pathway inhibitor 2 (TFPI2) secretion has been demonstrated to actively recruit and polarize microglia toward an immunosuppressive phenotype through activation of the cluster of differentiation 51 (CD51)-Signal Transducer and Activator of Transcription 6 (STAT6) signaling pathway [17].

Microglia, furthermore, are more prevalent in IDH-mutant gliomas, and higher levels of CD68-, CD163-, and cluster of differentiation 206 (CD206)-positive glioma-associated microglia and macrophages correlate with prolonged survival in GBM patients without the genetic mutation that involves the substitution of an arginine residue [R] with a histidine residue [H] at codon 132 (R132H) in the IDH1 gene, suggesting that modulation of these immune populations could have therapeutic benefits [5].

4.2 Bone marrow-derived macrophages (BMDMs)

A comprehensive study has analyzed the TME of the brain and demonstrated significant enrichment of tumor-associated myeloid cells/macrophages (TAMs) between glioma subtypes and brain metastasis [4]. BMDMs are predominantly localized to perivascular and perinecrotic regions of the tumor parenchyma of glioma [5, 11]. These cells suppress adaptive immunity, promote tumor growth, and contribute to neoangiogenesis.

Macrophage polarization is currently considered to be a major mechanism of escape from immune control of cancer growth [7]. Immunohistochemical studies have shown that in gliomas, most TAMs display anti-inflammatory M2-like polarization, marked by high CD163 and CD206 expression and low inducible nitric oxide synthase (iNOS) and cluster of differentiation 80 (CD80) levels.

Classically activated M1 macrophages are characterized by the expression of high levels of pro-inflammatory cytokines, like tumor necrosis factor (TNF), interleukin 1 (IL-1), interleukin 8 (IL-8), and interleukin 12 (IL-12), which influence lymphocyte activation, proliferation, and generation of effector cells; upregulation of cell surface molecules necessary for antigen presentation (including major histocompatibility complex (MHC) class II and costimulatory molecules like CD80 and cluster of differentiation 86 (CD86); promotion of T helper type 1 (Th1) response; and strong tumoricidal potential [7, 17].

In contrast, alternatively activated M2 macrophages are involved in promoting tissue remodeling and tumor progression, displaying immunosuppressive functions via both the production of the immunosuppressive cytokines IL-10, interleukin 6 (IL-6), interleukin 23 (IL-23), and TGF- β and the downregulation of MHC class II, CD80, and CD86 [7, 17, 18].

TAMs play a pivotal role in GBM progression by facilitating creatine biosynthesis and transfer within hypoxic tumor niches [5]. Solute carrier family 6 member 8 (SLC6A8)-mediated creatine uptake supports GBM cell survival and stemness under metabolic stress [7].

The recruitment of TAMs to the tumor site is driven by specific chemotactic signals, including CSF-1/CSF-1R, C-X-C chemokine receptor type 4 (CXCR4)/C-X-C motif chemokine ligand 12 (CXCL12), and hepatocyte growth factor (HGF)/mesenchymal-epithelial transition (MET) pathways [7, 18]. Periostin (POSTN) is secreted by glioblastoma stem cells (GSCs), and it acts as a chemoattractant for circulating monocytes that infiltrate the tumor and differentiate into M2-like macrophages via integrin $\alpha\text{v}\beta 3$ signaling [5]. These M2 macrophages establish an immunosuppressive niche, promoting GBM progression [5]. Targeting macrophage recruitment and polarization via CSF-1R or C-C chemokine receptor type 2 (CCR2) inhibitors is currently under investigation as a therapeutic approach.

Additional work has highlighted the role of macrophages in inducing a mesenchymal-like state in GBM, showing that macrophage-derived oncostatin M (OSM) interacts with its receptor on glioma cells, which in turn promotes the mesenchymal state via Signal Transducer and Activator of Transcription 3 (STAT3) signaling [17]. The Janus kinase 2 (JAK2)/STAT3 pathway regulates many cellular processes in GBM, including cell survival, proliferation, invasion, anti-apoptosis, and immune evasion, making STAT3 a possible target for therapy [7].

Mechanisms by which TAMs facilitate the intravasation of tumor cells are mediated via molecular interactions that involve a paracrine loop in which epidermal growth factor (EGF), produced by TAMs, increases the invasiveness and migration of cancer cells expressing the EGF receptor (EGFR) [7].

A reprogramming of myeloid cells toward an immature, immunosuppressive phenotype, which diminishes antigen presentation and facilitates tumor immune evasion, is further induced by IDH mutations [5]. For instance, tryptophan metabolism through the kynurenine pathway promotes immunosuppressive myeloid cell states in IDH-mutant gliomas, contributing to the circumvention of immune responses [4].

A substitution to methionine in the variant of histone-3 (H3.3) at lysine 27 (H3.3K27M) in diffuse midline gliomas are strongly associated with increased TAM infiltration, heightened immune suppression, and reduced survival compared to other histone variants [5].

These immune-related biomarkers may have the potential to predict patient outcomes and therapy responsiveness and thus have inspired numerous microenvironment-targeted therapies in multiple tumors [12]. For example, genetic and

pharmacological interventions targeting chemokine receptors such as C-C chemokine receptor type 1 (CCR1) and C-C chemokine receptor type 5 (CCR5) have demonstrated efficacy in disrupting macrophage recruitment and enhancing anti-tumor immune activity, ultimately extending survival [18].

4.3 Neutrophils

In the glioma microenvironment are present other myeloid cells, such as neutrophils. The role of these leukocytes in the tumor context was not investigated until a few years ago; they were considered innocent bystanders in the tumor stroma. On the contrary, emerging evidence indicates that neutrophils can play an important role in tumors, having both pro-tumoral and anti-tumoral functions [14]. Neutrophil phenotype is altered within the brain TME; they are more abundant in human brain metastases (BrMs) than in primary gliomas and are also present within the tumor lesions [19]. Brain tumor-associated neutrophils (TANs) differ significantly from blood neutrophils and have a prolonged lifespan and immunosuppressive and pro-angiogenic capacity [14].

TANs exhibit a distinct inflammatory gene signature, driven by a combination of soluble inflammatory mediators, such as tumor necrosis factor alpha (TNF- α) and ceruloplasmin, which are more pronounced in TANs from brain metastases than gliomas. This signature becomes more pronounced in BrMs but not in gliomas. Spatial TME analyses reveal the association of TANs with programmed death protein-1 (PD-1) + CD8+ T cells and endothelial cells [19].

The number of circulating and infiltrating neutrophils correlates with the severity of the disease, tumor growth (via the production of arginase I (ARG1)), and resistance to antiangiogenic therapies, indicating a prognostic and possible pro-tumoral role. Baseline neutrophil count in blood, in fact, predicts bevacizumab efficacy in GBM patients and the acquired resistance to anti-VEGF (vascular endothelial growth factor) therapy. Activation of neutrophils, measured by increased surface expression of CD11b, is also an early predictor of tumor progression [14]. In the tumor tissue, like macrophages, neutrophils are present in different polarized states: the neutrophil subtype (N1) anti-tumoral phenotype induced by interferon beta (IFN- β) stimulation and the neutrophil subtype (N2) pro-tumoral phenotype induced by TGF- β and granulocyte colony stimulating factor (G-CSF) stimulation [19].

Recent data indicate that neutrophils can be activated to directly kill tumor cells or to orchestrate the anti-tumoral response [11]. TANs also produce MMPs that facilitate ECM degradation and tumor invasion. Neutrophils are recruited to the tumor site by C-X-C motif chemokine ligand 8 (CXCL8) produced by Fas ligand (FasL) triggering on glioma cells. Immunosuppressive neutrophils are also recruited by the chemotactic agent macrophage migration inhibitory factor (MIF) produced by glioma cancer stem cells. At the tumor site, neutrophils secrete elastase that can aid glioma infiltration and can directly induce the proliferation of GBM-initiating cells by producing S100 calcium-binding protein 4 (S100A4) [14, 19].

Moreover, mutant IDH1 glioma tumors that have longer survival compared to wild-type IDH1 have reduced neutrophil infiltration [4]. Consideration should be given to the possibility of improving monoclonal antibody therapies by combining them with treatments that promote the differentiation of neutrophils into an anti-tumor phenotype with higher ADCC (antibody-dependent cellular toxicity) activity. Interestingly, two treatments, type I interferons (IFNs) and TGF- β targeting, which can promote neutrophil differentiation toward an anti-tumoral phenotype, are involved in glioma regression [14].

Targeting neutrophil recruitment (e.g., C-X-C motif chemokine receptor 2 (CXCR2) inhibitors) may offer new therapeutic angles. Finally, the ability of neutrophils to migrate into the inflamed brain can be exploited to deliver anti-cancer drugs in gliomas after surgery, reducing the regrowth of tumors [14].

4.4 Myeloid-derived suppressor cells (MDSCs)

A class of immune cells, able to differentiate into immunosuppressive TAMs under the effect of hypoxia and hypoxia-inducible factor 1- α (HIF-1 α), called MDSCs, is present in elevated numbers in the bone marrow, blood, and spleen of glioma tumor patients [11].

MDSCs are a minority (4–8%) of CD45+ cells in GBM and play a central role in fostering immune evasion and propelling tumor progression, angiogenesis, invasion, and metastasis. Tumors can secrete specific chemokines, such as CXCR4-CXCL12, CXCR2-CXCL5/8 (C-X-C motif chemokine ligand 5/C-X-C motif chemokine ligand 8, and CCR2 (C-C chemokine receptor type 2)-CCL2 (C-C motif chemokine ligand 2), to facilitate the recruitment of MDSCs [5]. Thus, heightened infiltration within the TME of MDSCs is associated with a poorer prognosis [10].

MDSCs express surface markers such as cluster of differentiation 11b (CD11b), vascular non-inflammatory molecule 2 (VNN2), and Granulocyte Receptor-1 (Gr-1) but lack the expression of MHC class II antigens and are a mixed population of monocytic and granulocytic cells [7].

MDSCs can be categorized into two types: monocytic myeloid-derived suppressor cells (M-MDSCs) and polymorphonuclear (PMN)-MDSCs [5]. In high-grade IDH-mutated gliomas, PMN-MDSCs are the predominant subset [4]. Currently, cluster of differentiation 49d (CD49d) is considered a specific marker for M-MDSCs, while lectin-like oxidized low-density lipoprotein receptor 1 (LOX1) is becoming a novel specific marker for PMN-MDSCs [7].

MDSCs exert their immunosuppressive effects by inhibiting the release of inflammatory factors and activating immunosuppressive cells; by interacting with tumor-associated B cells or regulatory B cells (Bregs) through extracellular vesicles (EVs); by transmitting programmed cell death ligand-1 (PD-L1) to regulate B-cell function and consequently inhibiting the typical immune function of CD8+ T cells. MDSCs are involved in various pathways, including Toll-like receptor (TLR) signaling, certain pro-inflammatory cytokines (like interleukin 13 (IL-13), interleukin 4 (IL-4), prostaglandin E2 (PGE2), IFN- γ , and interleukin 1 beta (IL-1 β)), activation of nuclear factor kappa B (NF- κ B) signals facilitating inducible nitric oxide synthase 2 (iNOS2) expression, and exosome secretion. Additionally, ROS, peroxynitrite (PNT), inositol-requiring enzyme 1 α (IRE1 α), human-activating transcription factor 6 (ATF6), and activation of endoplasmic reticulum stress (ERS), which increase the expression of ERS-related proteins (C/EBP Homologous Protein [CHOP], LOX1, Death Receptor 5 [DR5]), interleukin 6 (IL-6), CCAAT/enhancer-binding protein beta (C/EBP β), and phosphorylated Signal Transducer and Activator of Transcription 3 (pSTAT3), further enhance MDSC recognition and targeting of immune cells in the TME [5, 18].

Conversely, TNF- α and IFN- γ can promote the formation of a pro-inflammatory phenotype in the GBM microenvironment by reducing MDSC numbers. This process is activated by the Janus kinase-Signal Transducer and Activator of Transcription (JAK/STAT) signal, inducing Interferon Regulatory Factor 1 (IRF1) downregulation, promoting the secretion of PD-L1, and altering the immune-escape microenvironment [5]. However, the upregulated expression of FAT atypical cadherin 1 (FAT1)

enhances IL-1 β , IL-10, PD-L1, IL-6, and HIF-1 α secretion through the Activator Protein-1 (AP-1) signal [11].

Understanding the interplay between GBM and MDSCs provides a compelling basis for therapeutic interventions. If aimed at blocking MDSC recruitment to tumors by targeting specific chemokines or cytokines, therapeutic interventions may have limited impact; instead, a potentially more effective approach could involve targeting specific chemokine receptors, as certain receptors can interact with multiple chemokines [10].

5. Adaptive immune resistance and lymphoid cells

The adaptive immune response in gliomas is markedly impaired. Effector T cells, especially cytotoxic CD8⁺ T lymphocytes, are typically scarce within the TME.

Macrophages induce a series of changes that cause the production of an immunosuppressive environment, favoring tumor growth; in fact, microglia/macrophages cannot induce T-cell proliferation to limit tumor growth [7]. The consequences are T-cell inhibition/activation, proliferation, anergy, and apoptosis [15].

In a mouse model of glioma, both tumor-infiltrating macrophages and microglia were reported to express high levels of PD-L1 and accounted for 50% of the FasL-expressing cells, enough to be considered a major cause of the induced apoptosis of lymphocytes [20].

T-cell activation and proliferation in gliomas are inhibited by the production of immunosuppressive molecules, such as IL-10, TGF- β 1 [85-87], and IL-6 [88]. IL-10 also downregulates the HLA-DR (Human Leukocyte Antigen D-Related) expression, an MHC (major histocompatibility complex) class II cell surface receptor expressed by monocytes. HLA-DR associates with and presents peptidic antigens to the immune system to regulate T (helper) cell responses [21].

The inhibition of T-cell activation and expansion as well as B-cell anergy also results from the absence of costimulatory molecules on microglia/macrophages, such as CD86, cluster of differentiation 80 (CD80), and cluster of differentiation 40 (CD40). Those present often express exhaustion markers such as PD-1, lymphocyte activation gene 3 (LAG-3), and T-cell immunoglobulin and mucin domain 3 (TIM-3), indicating functional anergy. On the other hand, human leukocyte antigen G (HLA-G), human leukocyte antigen E (HLA-E), and PTGER2 (Prostaglandin E Receptor 2) expression leads to reduced activity of NK cells, cytotoxic T lymphocytes (CTLs), and T-cell proliferation [7]. Moreover, the inhibitory effects of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) act to inhibit T-cell effector function and enhance the inhibitory activity of Tregs in a murine glioma model [22].

IDH-mutant gliomas tend to have even lower lymphocyte infiltration compared to IDH-wild-type tumors [4]. Moreover, these tumors often exhibit hypermethylation of immune-related gene promoters, including PD-L1, resulting in reduced checkpoint ligand expression and diminished immune recognition [20].

Other actors can play opposing roles: data concerning lymphocyte infiltrate and prognosis of gliomas indicate a beneficial role of immune T-cell infiltration inside the tumor in GBM patients, despite multiple tumor immune-escape mechanisms. Indeed, increased CD3⁺ T-cell infiltration is associated with prolonged survival independent of age [7]. Overall, the lack of robust T-cell infiltration and the dominance of suppressive lymphoid populations severely limit the effectiveness of adaptive immunity in glioma patients.

Single-cell RNA sequencing has revealed that glioma-infiltrating T cells co-express cytotoxic markers and NK-cell genes, suggesting the presence of hybrid T-cell states with distinctive functional properties. These T cells express cluster of differentiation 161 (CD161), an inhibitory receptor that reduces their cytotoxic potential through interaction with CLEC2D (C-type lectin domain family, 2 member D) on tumor and myeloid cells. Genetic or antibody-mediated blockade of CD161 significantly enhances T-cell-mediated killing of glioma cells. Furthermore, tumor-derived fibrinogen-like protein 2 (FGL2) suppresses the differentiation of CD103⁺ dendritic cells (DCs), thereby impairing CD8⁺ T-cell-mediated immune responses against GBM [5]. Lad et al. discovered that GBM recruits immature neutrophils from the skull bone marrow, which differentiate into antigen-presenting hybrid neutrophils capable of activating T cells [23]. The study identified a unique dendritic-like TAN phenotype that suppressed GBM growth through MHC-II-dependent T-cell activation mechanisms [5].

T helper cells are skewed toward a T helper type 2 (Th2) phenotype, secreting anti-inflammatory cytokines that further suppress cytotoxic responses. Tregs, characterized by Forkhead box P3 (FOXP3) expression, are frequently enriched and contribute to immune evasion by suppressing CD8⁺ T-cell proliferation and dendritic cell activation [18].

6. Molecular drivers of immunosuppression

The immunosuppressive glioma microenvironment, particularly within the context of cell-mediated immunity, is orchestrated by multiple molecular mediators [5]. The systemic immunosuppression is driven by the overexpression of soluble factors by glioma cells. Among these, TGF- β plays a central role by inhibiting T-cell proliferation and NK-cell cytotoxicity while promoting Treg differentiation and M2 polarization of macrophages. IL-10, secreted by tumor cells and TAMs, suppresses antigen presentation and cytokine production by dendritic cells. Arginase and indoleamine 2,3-dioxygenase (IDO) contribute to the metabolic suppression of T cells through the depletion of arginine and tryptophan, respectively. Prostaglandin E2 (PGE2) and adenosine signaling further dampen T-cell activation. It occurs, therefore, as a decreased T-cell responsiveness to pro-inflammatory signals and ineffective presentation of tumor antigens by antigen-presenting cells (APCs). Additionally, the same mediators drive the recruitment of immunosuppressive cells, such as regulatory T cells and myeloid suppressor cells, to the TME. Moreover, corticosteroids (often used in glioma patients to diminish cerebral edema) are immunosuppressive, and temozolomide chemotherapy and radiotherapy can trigger prolonged lymphopenia. Several immunosuppressive alterations do occur within the glioma microenvironment [14]. Glioma cells can downregulate their own MHC-I (major histocompatibility complex class I) complexes, thus becoming “invisible” to the adaptive immune system, as well as increase the expression of immune checkpoint regulators, such as PD-1 and cluster of differentiation 47 (CD47), in response to environmental stress and oncogenic signaling [10].

Epigenetic changes, including promoter methylation and histone modification, play a role in regulating immune-related gene expression, especially in IDH-mutant gliomas [4].

Together, these molecular pathways form a robust immunosuppressive network that poses significant barriers to immunotherapy.

7. Current immunotherapeutic approaches

The idea of tumor immunotherapy is to deploy the immune system as a tool to treat cancer. Immunotherapy offers great hope for a cure in cancer treatment by reeducating and harnessing the patient's immune response to the tumor. Comprehensive analysis of the brain TME suggests that modulating the diverse phenotypes of immune cells, rather than depleting them, could enhance the effectiveness of immunotherapies for brain cancers [5]. Current immunotherapeutic strategies used to treat cancer are mainly based on ICIs, therapeutic vaccines, adoptive cell therapy, monoclonal antibodies, and oncolytic viruses [24].

ICIs consist of monoclonal antibodies that neutralize immunosuppressive signaling and enhance immune responses against tumor cells by targeting costimulatory and inhibitory molecules, which can regulate the activation and effector functions of T lymphocytes [9]. ICIs such as pembrolizumab and nivolumab are anti-programmed cell PD-1; durvalumab, atezolizumab, and avelumab are anti-PD-L1; and ipilimumab is anti-CTLA-4 [13]. Particularly, PD-1/PD-L1 immunotherapy has been confirmed to be successful [24]. Toll-like receptor (TLR) agonists can be used as immunomodulators to avoid dose accumulation, boost the efficiency of other therapies, and, by upregulating PD-1, reinforce delayed immune checkpoint resistance against PD-1/PD-L1 inhibition [21]. Therefore, the use of TLR agonists can lead to PD-L1 overexpression, which, in turn, enhances the efficacy of immune checkpoint inhibitors and triggers potent anticancer immune responses [20, 24].

Inhibiting the JAK/STAT pathway and the vascular endothelial growth factor receptor (VEGFR) is effective in transforming “cold tumors” into “hot tumors” [12].

ICIs, previously deemed less effective in GBM, may warrant reevaluation of their utility when used in conjunction with these therapies [13].

One of the major translational barriers for TAM-targeting therapies in GBM is effective delivery across the BBB. Several delivery strategies include nanoparticle and liposome-based formulations, which can improve BBB traversal and target specificity, as well as focused ultrasound-assisted BBB disruption, which transiently increases vascular permeability in a controlled and localized manner [18]. Immunomodulatory cytokines like TNF- α and interferon alpha (IFN- α) can traverse the BBB and have been demonstrated to be effective at reversing GBM-induced immunosuppression [10].

Multiple strategies against gliomas to overcome immunosuppression and boost anti-tumor immune responses are now under evaluation in clinical trials, including vaccination with tumor-specific or tumor-associated peptides (or with the administration of autologous APCs pulsed with such peptides), immunomodulatory monoclonal antibodies that target T-cell inhibitory signaling pathways to enhance physiological anti-tumor responses, and adoptive T-cell transfer, which involves ex vivo clonal expansion or genetic engineering of T cells [13].

Vaccination strategies targeting tumor-specific antigens (e.g., epidermal growth factor receptor variant III (EGFRvIII), IDH1 R132H) have been tested in early-phase trials, with modest results. More recently, neoantigen-based personalized vaccines have emerged as a promising but complex approach. Cell-based therapies, including chimeric antigen receptor (CAR) T cells targeting interleukin 13 receptor alpha 2 (IL13R α 2) or EGFRvIII, face challenges related to trafficking, persistence, and immunosuppression in the TME. Identification of novel therapeutic targets will emerge through advances in single-cell and spatial transcriptomics; these will further elucidate glioma heterogeneity [5]. Emerging modalities such as engineered immune

cells, oncolytic viruses, and therapeutic immune modulatory microRNAs (miRs), and nanotechnology-based drug delivery hold promise for overcoming glioma's resistance mechanisms [17].

In addition to strategies aimed at T-cell activation, efforts were also made to target tumor-associated macrophages to inhibit glioma progression by myeloid cell reprogramming [14]. Inhibiting macrophage chemotaxis and the polarization of macrophages from a pro-tumoral (M2-like) to a pro-inflammatory (M1-like) phenotype is considered a promising approach for various types of tumors [17, 18]. Several approaches have been performed using CD40 agonists, phosphoinositide 3-kinase γ (PI3K γ) inhibitors, TLR or stimulators of interferon genes (STING) pathway activators, inhibitors of the CSF-1 receptor (cluster of differentiation 115 (CD115), or TGF- β blockers [18]. Inhibition of M-CSF has been identified as a significant therapeutic target for GBM in numerous studies and its anti-tumor effect was enhanced when it was combined with radiation therapy [12].

Key inhibitors aimed at reprogramming the TME are those of CSF-1R (e.g., (PLX3397 [pexidartinib]), BLZ945 [sotuletinib]), CXCR4 antagonists (e.g., AMD3100 [Plerixafor]), NOX-A12 [olaptosed pegol]), an RNA-based aptamer that binds to CXCL12 and blocks its interaction with the CXCR4 receptor and other molecular inhibitors. Crizotinib and Cabozantinib are multikinase inhibitors that target the MET receptor along with vascular endothelial growth factor receptor 2 (VEGFR2), rearranged during transfection (RET), and Anexelekto (AXL). Foretinib is an oral multikinase inhibitor targeting several receptor tyrosine kinases (RTKs), including MET, AXL, and VEGFR2. Onartuzumab (MetMAb) is a monoclonal antibody that targets the c-Met (c-mesenchymal-epithelial transition factor) receptor. Capmatinib is a small-molecule tyrosine kinase inhibitor that selectively targets the MET receptor [18].

The personalized and combinatorial strategies that integrate immunotherapy, metabolic modulation, and microenvironment-targeting agents will be future therapeutic approaches for glioma treatment [5].

8. Immunology in diagnostics

The application of immunology to the diagnostics of gliomas represents a rapidly evolving field today, with an increasing impact on clinical practice. This approach allows not only for refining diagnostic accuracy and speed but also for improving and facilitating the early identification of potential therapeutic targets, contributing to defining increasingly targeted and personalized treatment strategies. Immunohistochemical techniques, integrated with advanced digital technologies, enable detailed qualitative and morphological analysis of neoplasms, highlighting the presence and distribution of immune cell subpopulations and the expression of specific molecular markers.

This paragraph explores the immunological foundations that guide glioma diagnostics, focusing on the currently most used methodologies and the new opportunities made possible by the integration of digital technologies and intraoperative diagnostics. The goal is to provide an updated and consistent view of the potential offered by an increasingly accurate, timely, and personalized diagnostic approach.

Gliomas represent the most important primary brain tumors from a clinical perspective [3]; they are characterized by marked cellular, molecular, and immunological heterogeneity. The integration of immunology and diagnostics allows

for the study of neoplasia not only from a morphological point of view but also, as previously illustrated, the behavior of immune system cells present in the TME. Immunohistochemistry has been a turning point in the classification of brain tumors since 1993, with the release of the second edition of the WHO classification [2].

Applied immunology to diagnostics, using labeled antibodies and immunohistochemistry (IHC) techniques, allows for the specific identification of molecules expressed by tumor cells or infiltrating immune cells, providing fundamental support for traditional histopathological diagnosis and enabling useful therapeutic assessments such as the expression of PD-L1 [24], whose evaluation seems to take on new importance. In this context, digital technologies for diagnostics are also included, such as the VivaScope system, which allows for rapid and real-time assessments of tissue and cellular characteristics through confocal microscopy and immunohistochemistry. In the context of neuropathology, the diagnosis of gliomas has progressively evolved by integrating immunological approaches that allow for a more thorough delineation of the biological and functional profile of the neoplasm.

Immunohistochemistry represents a fundamental tool for identifying antigens specifically expressed by tumor cells, as well as for assessing markers associated with the immune response of the local microenvironment. Among the main markers used, Glial Fibrillary Acidic Protein (GFAP) is employed to confirm the glial origin of the tumor, particularly of the astrocytic or ependymal type. The IDH1 or isocitrate dehydrogenase 2 (IDH2) mutations are a molecular marker of significant diagnostic and prognostic value, widely used in the classification of gliomas according to the criteria of the World Health Organization (**Figure 2A**). The proliferative marker Ki-67 provides indications on the percentage of cell replication, proving useful in estimating the degree of aggressiveness of the tumor. The loss of expression of the alpha-thalassemia X-linked intellectual disability (ATRX) protein is frequently associated with somatic mutations in glial neoplasms and is clinically relevant, especially in low-grade diffuse gliomas. The overexpression of the tumor suppressor protein p53, which is involved in the regulation of the cell cycle and the response to DNA damage, is generally indicative of a potentially oncogenic mutation. Many of these markers are also useful in distinguishing non-neoplastic glial proliferation from neoplastic proliferation. Finally, the PD-L1 marker is used to evaluate the activation of immune checkpoint mechanisms, thus representing a potential target for immunomodulatory therapies (**Figure 2B**). The integration of these immunohistochemical markers into diagnostic practice contributes to the definition of personalized therapeutic strategies and to the improvement of patient prognosis.

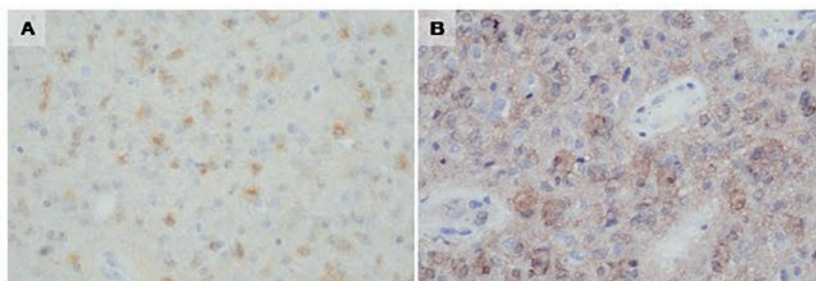


Figure 2. Expression of markers in glioma by immunohistochemistry. 40x microscope acquisitions. (A) Expression of the product of R132H-mutated IDH1 gene in astrocytoma grade 2. (B) Expression of the PD-L1 protein in GBM cancer cells. The antibody localizes on the cell membrane.

Early, some aspects of the immune response may become part of routine diagnostic practice, contributing to the definition of prognosis in patients with glioma.

The immunological profile of these neoplasms, characterized, for example, by a predominance of Treg, M2 polarization of macrophages, or reduced expression of PD-L1, has been associated with unfavorable clinical outcomes, suggesting the presence of an immunosuppressive microenvironment [2, 13]. Particularly, the gene expression of specific subtypes of immune cells appears to be closely related to the mutational status of IDH and the degree of tumor aggressiveness [4, 13].

In recent years, digital diagnostics have profoundly transformed the intraoperative context and the practice of histopathological reporting, bringing significant advantages in cases where the speed of diagnostic evaluation is essential, such as during the surgical removal of a neoplasm. Among the most advanced technologies, laser

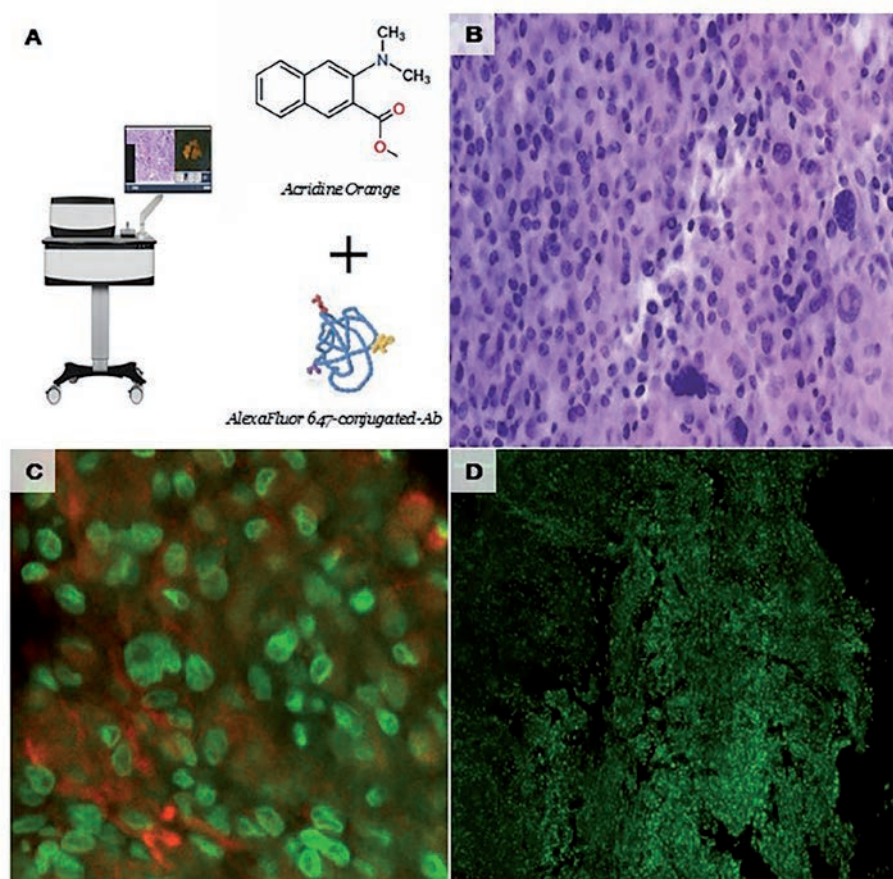


Figure 3.
 Instant diagnosis of high-grade glial neoplasia by immunohistochemistry carried out with VivaScope equipment. (A) Explicative schema of immunohistochemistry analysis by VivaScope instrument. A tumor sample is stained with Acridine Orange, then incubated with a fluorescent antibody to mark the protein of interest (e.g., AlexaFluor 647) and analyzed using VivaScope instrument. (B) Digital simulation of hematoxylin and eosin stain, observed after treatment with Acridine Orange. (C) Digital image of tumor sample immunolabeled with an AlexaFluor 647-conjugated anti-GFAP monoclonal antibody (Red-positive). (D) Digital image of tumor sample immunolabeled with an AlexaFluor 647-conjugated anti-IDH1 (mutant R132H) monoclonal antibody (Red-negative).

confocal microscopy systems stand out, capable of acquiring high-resolution images in real time on fresh tissues. These tools allow for immediate detailed morphological information, preceding the insights provided by traditional histological procedures (Figure 3).

The clinical advantage of equipment like the VivaScope instrument lies in its ability to guide surgical decisions in real time, increasing the safety and accuracy of biopsy sampling and potentially avoiding incomplete or unnecessary resections. Rapid immunofluorescent staining can become an essential tool for intraoperative immunophenotypic characterization, for example, enabling accurate differentiation during surgery between a primary neoplasm and a secondary one.

Figure 3 depicts the standard procedure and the case of a 69-year-old patient affected by brain neoplasia, in which the final diagnosis of wild-type GBM corresponds to that which can be made at that time using confocal technology [25, 26]. The procedure allows for the instant diagnosis of wild-type GBM.

Applied immunology in the diagnosis of gliomas continues to represent a fundamental and constantly evolving pillar. Despite advancements in knowledge and technology, the contribution of immunohistochemical analyses remains central, today enriched by the potential offered by digital diagnostics. The integration of innovative and traditional approaches allows for a more comprehensive and dynamic evaluation of the tumor, improving diagnostic accuracy and opening up new perspectives for a more precise prognosis and the definition of increasingly personalized therapeutic strategies.

9. Future perspectives

Innate immunity in brain tumors must be explored further to better understand the mechanisms of progression and growth of these neoplasms, and because immunotherapy may play a larger role in future therapeutic protocols [7].

As our understanding of glioma immunology deepens, several promising avenues are emerging. A combined analytic approach, which can evaluate both lymphocytic and macrophagic components, could help to stratify the patient population more effectively at the prognostic level [7]. Future efforts should focus on establishing more accurate correlations between genetic markers and microenvironmental/functional patterns to assist therapy settings and formulate prognostic evaluations [7, 12].

Patient stratification based on immune signatures, IDH status, and spatial immune profiling could guide more targeted interventions. The application of single-cell and spatial transcriptomics offers unprecedented resolution in dissecting the glioma immune microenvironment [4].

Novel targets, such as cluster of differentiation 47 (CD47) (“don’t eat me” signal), T-cell immunoglobulin and mucin domain 3 (TIM-3), and T-cell immunoreceptor with immunoglobulin (Ig) and ITIM (immune receptor tyrosine-based inhibitory motif) domains (TIGIT), are being investigated [13].

Additionally, epigenetic therapy to reverse immune gene silencing or metabolic reprogramming to enhance immune cell function is under early exploration. Engineered oncolytic viruses and nanoparticles offer platforms for delivering immunostimulatory agents directly into the tumor, bypassing systemic barriers. Finally, integrating immunotherapy with standard-of-care treatments in a rational, temporally optimized manner holds promise to unlock therapeutic synergy [10].

10. Conclusions

Gliomas remain one of the most challenging malignancies from an immunological standpoint. The complex interplay between tumor cells, immune infiltrates, and the surrounding stroma creates a formidable barrier to effective immune-mediated clearance.

However, growing insights into the glioma immune microenvironment and the molecular pathways driving immunosuppression are paving the way for new diagnostic and therapeutic opportunities. Precision immunotherapy, tailored to the molecular and immune profile of individual tumors, represents the future of glioma treatment.

Acknowledgements

The authors acknowledge financial support from the Ministry of Health, Italy—European Union—Next Generation EU—through the IDRA project named “Development Of Multiparametric Approach For New Instant Digital Pathology To Support Fast Diagnostic Precision And Appropriate Clinical Decision” (PNRR-POC-2022-12376531) in the PNRR-M6C2.2 “Investment 2.1. Enhancement and strengthening of biomedical research in the NHS”. CUP MASTER F83C22002490001.

The authors acknowledge Prof. Francesco Di Matteo (Fondazione Policlinico Universitario Campus Bio-Medico, Rome, Italy) and Prof. Anna Crescenzi (Sapienza University of Rome, Rome, Italy) for their scientific support.

Conflict of interest

The authors declare no conflict of interest.

Other declarations

This study was conducted in accordance with Declaration of Helsinki and approved by “Comitato Etico Sezione Area Centro - Regione Calabria” (Protocol n. 64 of 16 March 2023). Informed consent was obtained from all the subjects involved in the study.

The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s). IntechOpen and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Author details

Anna Bilotta¹, Deborah Dardano¹ and Giuseppe Donato^{1,2*}

1 “R. Dulbecco” University Hospital, Catanzaro, Italy

2 University “Magna Græcia” of Catanzaro, Catanzaro, Italy

*Address all correspondence to: gdonato@unicz.it

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] van den Bent MJ, Geurts M, French PJ, Smits M, Capper D, Bromberg JEC, et al. Primary brain tumours in adults. *The Lancet*. 2023;**402**:1564-1579
- [2] Kleihues P, Burger PC, Scheithauer BW. The new WHO classification of brain tumours. *Brain Pathology*. 1993;**3**(3):255-268
- [3] Torp SH, Solheim O, Skjulsvik AJ. The WHO 2021 classification of central nervous system tumours: A practical update on what neurosurgeons need to know—A mini review. *Acta Neurochirurgica*. 2022;**164**(9):2453-2464
- [4] Mehani B, Asanigari S, Chung HJ, Dazelle K, Singh A, Hannenhalli S, et al. Immune cell gene expression signatures in diffuse glioma are associated with IDH mutation status, patient outcome and malignant cell state, and highlight the importance of specific cell subsets in glioma biology. *Acta Neuropathologica Communications*. 2022;**10**(1):19
- [5] Jang HJ, Park JW. Microenvironmental drivers of glioma progression. *International Journal of Molecular Sciences*. 2025;**26**(5):2108
- [6] Schaff LR, Mellinghoff IK. Glioblastoma and other primary brain malignancies in adults. *Journal of the American Medical Association*. 2023;**329**(7):574
- [7] Guadagno E, Presta I, Maisano D, Donato A, Pirrone CK, Cardillo G, et al. Role of macrophages in brain tumor growth and progression. *International Journal of Molecular Sciences*. 2018;**19**(4):1005
- [8] Berghoff AS, Kiesel B, Widhalm G, Wilhelm D, Rajky O, Kurscheid S, et al. Correlation of immune phenotype with IDH mutation in diffuse glioma. *Neuro-Oncology*. 2017;**19**(11):1460-1468
- [9] Di Vito A, Donato A, Bria J, Conforti F, La Torre D, Malara N, et al. Extracellular matrix structure and interaction with immune cells in adult astrocytic tumors. *Cellular and Molecular Neurobiology*. 2024;**44**(1):54
- [10] Lin H, Liu C, Hu A, Zhang D, Yang H, Mao Y. Understanding the immunosuppressive microenvironment of glioma: Mechanistic insights and clinical perspectives. *Journal of Hematology and Oncology*. 2024;**17**(1):31
- [11] Roesch S, Rapp C, Dettling S, Herold-Mende C. When immune cells turn bad—Tumor-associated microglia/macrophages in glioma. *International Journal of Molecular Sciences*. 2018;**19**(2):436
- [12] Matsuzaki H, Pan C, Komohara Y, Yamada R, Yano H, Fujiwara Y, et al. The roles of glioma-associated macrophages/microglia and potential targets for anti-glioma therapy. *Immunological Medicine*. 2025;**48**:24-32
- [13] Vismara MFM, Donato A, Malara N, Presta I, Donato G. Immunotherapy in gliomas: Are we reckoning without the innate immunity? *International Journal of Immunopathology and Pharmacology*. 2019;**33**:1-5
- [14] Massara M, Persico P, Bonavita O, Poeta VM, Locati M, Simonelli M, et al. Neutrophils in gliomas. *Frontiers in Immunology*. 2017;**8**(OCT):1349
- [15] Presta I, Vismara M, Novellino F, Donato A, Zaffino P, Scali E, et al. Innate immunity cells and the

neurovascular unit. *International Journal of Molecular Sciences*. 2018;**19**(12):3856

[16] Douyère M, Chastagner P, Boura C. Neuropilin-1: A key protein to consider in the progression of pediatric brain tumors. *Frontiers in Oncology*. 2021;**11**:665634

[17] Mignogna C, Signorelli F, Vismara MFM, Zeppa P, Camastra C, Barni T, et al. A reappraisal of macrophage polarization in glioblastoma: Histopathological and immunohistochemical findings and review of the literature. *Pathology, Research and Practice*. 2016;**212**(6):491-499

[18] Lee C, Lee J, Jeong M, Nam D, Rhee I. Emerging strategies for targeting tumor-associated macrophages in glioblastoma: A focus on chemotaxis blockade. *Life Sciences*. 2025;**376**:123762

[19] Maas RR, Soukup K, Fournier N, Massara M, Galland S, Kornete M, et al. The local microenvironment drives activation of neutrophils in human brain tumors. *Cell*. 2023;**186**(21):4546-4566. e27

[20] Mohammadi Y, Kavini E, Ahmadvand S, Dehghanian A, Ghaderi A. Prognostic significance of PD-L1 and CD45RO+ cells in glioblastoma: The modulating role of MMR status. *Journal of Neuroimmunology*. 2025;**406**:578669

[21] Norollahi SE, Babaei K, Rashidy-pour A, Yousefi B, Baharlou R, Far BF, et al. Role of the TLR signaling pathway in the pathogenesis of glioblastoma multiforme with an emphasis on immunotherapy. *Biochemistry and Biophysics Reports*. 2025;**43**:102149

[22] Wei Y, Wu X, Bao H, Xiao Y, Diao H, Liu S, et al. The differences in molecular

profiles and survival outcomes between early-onset and late-onset glioblastoma multiforme. *Genes & Diseases*. ScienceDirect; 2025:101638

[23] Lad M, Beniwal AS, Jain S, Shukla P, Kalistratova V, Jung J, et al. Glioblastoma induces the recruitment and differentiation of dendritic-like “hybrid” neutrophils from skull bone marrow. *Cancer Cell*. 2024;**42**(9):1549-1569.e16

[24] Wu J, Wang N. Current progress of anti-PD-1/PDL1 immunotherapy for glioblastoma (review). *Molecular Medicine Reports*. 2024;**30**(6):221

[25] Krishnamurthy S, Sabir S, Ban K, Wu Y, Sheth R, Tam A, et al. Comparison of real-time fluorescence confocal digital microscopy with Hematoxylin-eosin-stained sections of Core-needle biopsy specimens. *JAMA Network Open*. 2020;**3**(3):e200476

[26] Stigliano S, Crescenzi A, Taffon C, Covotta F, Hassan C, Antonelli G, et al. Role of fluorescence confocal microscopy for rapid evaluation of EUS fine-needle biopsy sampling in pancreatic solid lesions. *Gastrointestinal Endoscopy*. 2021;**94**(3):562-568.e1

Immunotherapy in the Treatment of Melanoma

*Areti Katsouda, Thomas Rallis, Apostolos Gogakos,
Vasileios Theocharidis, Thomas Kanteres, Myrtyw Tzinevi,
Ioannis Passos and Maria Mironidou-Tzouveleki*

Abstract

Melanoma, an aggressive type of skin cancer, has historically posed significant therapeutic challenges when diagnosed in an advanced stage. The advent of immunotherapy over the recent decades has fundamentally transformed treatment strategies for melanoma. This chapter explores the evolution of immunotherapeutic approaches for cutaneous melanoma and their mechanism of action, focusing mainly on immune checkpoint inhibitors (ICIs), such as CTLA-4, PD-1, and PD-L1 inhibitors. Immunotherapy agents, such as ipilimumab, pembrolizumab, and nivolumab, have demonstrated substantial improvement of overall and progression-free survival in clinical trials, while their combination regimens offer enhanced efficacy at the cost of increased toxicity. Emerging modalities, such as intralesional therapies (T-VEC), personalized mRNA vaccines, and tumor-infiltrating lymphocyte (TIL) therapy, are currently being tested for their potential to overcome the resistance mechanisms of melanoma against ICIs and enhance the antitumor response. Immune-related adverse events (irAEs) remain a major concern in melanoma immunotherapy, necessitating careful patient selection as well as multidisciplinary management. The role of tumor microenvironmental (TME) factors and predictive biomarkers is also under consideration so that personalized immunotherapy may emerge in clinical practice. Despite the remarkable progress of immunotherapy agents and regimens, challenges continue to persist, including resistance, toxicity, and increased treatment cost. Thus, there is still a need for ongoing research and broader global access, along with the integration of novel combination strategies to further improve treatment outcomes. Immunotherapy for melanoma has shifted its prognosis from a fatal diagnosis to a manageable chronic condition, even in advanced cases, being one of the most important advances in modern oncology.

Keywords: melanoma, immunotherapy, immune checkpoint inhibitors, immune-related adverse events, immunotherapy resistance, tumor microenvironment

1. Introduction

Melanoma is one of the most biologically complex and aggressive forms of skin cancer, accounting for the majority of deaths from cutaneous cancer. Globally, the

incidence of melanoma has risen steadily over the past several decades, particularly in fair-skinned populations with high ultraviolet (UV) exposure. According to estimates from 2020, over 325,000 new cases of melanoma are diagnosed around the world each year, resulting in more than 57,000 related deaths [1–3]. Melanoma is a significant public health challenge in many regions, including Australia, the USA, and parts of Europe, where campaigns focused on public awareness have been implemented and various preventative strategies have been applied [2, 4, 5]. However, if the current trends continue, it is estimated that by 2040, there will be about 510,000 new melanoma cases and 96,000 deaths [1].

Cutaneous melanoma consists of a group of pigmented tumors that arise from melanocytes of the skin. These cells derive from the neural crest and commonly migrate to the skin and hair follicles during fetal development. They are known for their ability to produce melanin (a UV-absorbing polymer) that provides the pigment to the surrounding keratinocytes. The malignant transformation of the melanocytes is most likely initiated by UV light, which induces DNA mutations [6, 7].

Clinically, melanoma is mainly known for its resistance to conventional treatments in later stages, as well as its high metastatic potential. Early-stage melanomas may be cured with surgical excision; however, the outcomes are significantly worse once it has metastasized. The presentation and progression of the disease are heterogeneous and influenced by a complex interplay of genetic mutations and the immune response [5, 6].

Historically, the therapeutic landscape for advanced melanoma was bleak. Surgery has remained the cornerstone of early-stage management, with a 5-year survival rate of approximately 94–98% when diagnosed at the localized stage (according to the surveillance, epidemiology, and end results program (SEER) staging) [6]. However, metastatic disease had been treated mainly with nonspecific cytotoxic chemotherapy agents in the past, which resulted in limited efficacy and poor prognosis [8–10]. In the early 2010s, targeted therapies started emerging, offering improved progression-free survival for patients. However, these therapies were limited by the development of resistance, along with the relatively short-lived responses.

Immunotherapy emerged as a transformative approach in the treatment and management of melanoma. Melanoma is one of the most immunogenic of human malignancies and is characterized by a high tumor mutational burden (TMB) along with frequent immune cell infiltration. This led researchers to observe spontaneous tumor regression and responses to immune-modulating agents, including interleukin-2 (IL-2), paving the way for immunotherapy treatment for malignant melanoma [11, 12]. In the 1990s, high-dose IL-2 proved effective in metastatic melanoma, featuring an objective response rate (ORR) of 16%. However, from a long-term perspective, these immune reactions could not last, while a few people experienced intolerable adverse effects [13].

Over the past decade, the development of new immunotherapy agents and the clinical implementation of immune checkpoint inhibitors (ICIs), mainly targeting Cytotoxic T-Lymphocyte-Associated Antigen 4 (CTLA-4), Programmed Cell Death Protein 1 (PD-1), and Programmed Death-Ligand 1 (PD-L1), have significantly altered the trajectory of melanoma therapy. Overall survival (OS) has shown unprecedented improvement with the clinical implementation of ipilimumab, nivolumab, and pembrolizumab, especially among those patients with advanced stages of malignancy. These breakthroughs in melanoma treatment have not only redefined the standard of care but have also provided proof of concept for immunotherapy for other types of malignancies [7–9].

This chapter will explore the mechanisms, clinical applications, and ongoing challenges of immunotherapy as a treatment option, especially ICIs, in malignant melanoma [7, 11, 12].

2. Immunological basis of melanoma

Melanoma is noted for its exceptional immunogenicity, resulting in a high response to immunotherapy, mainly due to its distinct biological features, such as the abundant neoantigen formation, high mutation burden, reactive tumor microenvironment (TME), and a dynamic interaction with the immune system, including both immune activation and evasion mechanisms [14–18].

The high TMB is one of the main reasons that melanoma is so immunogenic, and it is primarily driven by the DNA damage caused by UV radiation. This damage leads to the generation of tumor-specific neoantigens, mutated proteins recognized as foreign by the immune system [19]. These neoantigens may stimulate CD4⁺ and CD8⁺ T-cell responses, forming the basis for an effective antitumor immunity [20].

Chronic inflammation, along with immune editing, usually shapes the melanoma TME, a complex network of tumor, immune, and stromal cells, including dense infiltrates of TILs, often used as a marker of preexisting antitumor immunity. In literature, high levels of TIL cells have been correlated with improved survival outcomes [19], and they may be utilized in other therapeutic strategies, such as adoptive T-cell transfer [21]. However, despite the high immunoreactivity of melanoma, the TME also features strong immunosuppressive forces, such as myeloid-derived suppressor cells (MDSCs), regulatory T-cells (Tregs), and immunosuppressive cytokines. These elements impair T-cell activation while facilitating tumor persistence [22].

At the same time, melanoma cells use a wide variety of strategies to bypass immune system surveillance, including the downregulation of antigen presentation machinery (e.g., loss of human leukocyte antigen (HLA) molecules) and the upregulation of immune checkpoints, such as PD-L1, resulting in binding to PD-1 on T-cells and inhibiting their normal function. Furthermore, melanoma cells develop a wide tumor heterogeneity through the selection of certain subclones with reduced immunogenicity to thrive [23]. These immune escape strategies can significantly impair the effectiveness of the immune response, rendering them major targets of modern therapies. This is mainly the reason why ICIs have been so successful in melanoma treatment, as they aim to restore T-cell function by blocking CTLA-4 or PD-1/PD-L1 pathways [12, 22, 24].

Perhaps the most direct mechanism of immune evasion is the downregulation or loss of major histocompatibility complex (MHC) class I molecules, which are essential for CD8⁺ T-cell recognition of tumor antigens. Melanoma cells can also carry epigenetic silencing or mutations in components of the antigen-processing machinery, like TAP1 and β 2-microglobulin, making them “invisible” to cytotoxic T-lymphocytes (CTLs) [19].

Melanoma cells may also take advantage of immune checkpoint pathways by upregulating ligands like PD-L1, which bind to PD-1 receptors on activated T-cells and inhibit their cytotoxic function. This process results in T-cell exhaustion, a dysfunctional state characterized by reduced cytokine production, cytolytic activity, and proliferative capacity [24]. The high levels of PD-L1 expression observed in malignant melanoma tumors have been correlated with a suppressive immune environment, especially in “inflamed” tumors with TIL infiltration [19, 22].

3. Checkpoint inhibitors in melanoma

The implementation of ICIs in melanoma treatment has been a breakthrough, providing additional treatment options and strategies with a good safety profile and notable effectiveness, offering improved overall survival—especially in advanced cases. These agents enable a durable and robust immune response against malignant melanoma cells through T-cell activation and function. The main immune checkpoints targeted in melanoma treatment strategies are CTLA-4 and PD-1, along with their ligand PD-L1.

3.1 CTLA-4 inhibition: Ipilimumab®

Ipilimumab® is a fully humanized IgG1 monoclonal antibody that blocks CTLA-4 [25, 26]. In the late 1980s, Brunet *et al.* described CTLA-4 as a 223–amino-acid protein that belongs to the immunoglobulin superfamily [27]. It is mainly expressed on activated lymphocytes and is co-induced along with T-cell-mediated cytotoxicity [28]. A few years later, the human homolog of the gene was cloned [13].

3.1.1 Mechanism of action

CTLA-4 is an immune checkpoint receptor found primarily on activated T-cells attempting to mount an immune response and on Tregs, which help suppress immune activity and maintain immune balance [26, 29].

In essence, CTLA-4 functions as the immune system's braking mechanism. Upon antigen recognition, a T-cell anticipates a second signal from antigen-presenting cell (APC) molecules, specifically the B7 family proteins (CD80 and CD86). Normally, CD28 on T-cells binds to B7 to provide this activating signal. However, CTLA-4 competes with CD28 for B7 binding, leading to inhibition of the activation signal and reduced T-cell activity. This interaction mainly takes place in lymphoid organs, where naïve T-cells first encounter antigens [13, 26, 29, 30].

Ipilimumab® blocks CTLA-4's ability to bind B7, removing the “brake” on immune responses. This allows CD28 to bind B7 effectively, promoting stronger and sustained activation and proliferation of T-cells, which can then attack melanoma cells.

3.1.2 Clinical evidence

Ipilimumab® was developed based on the above mechanisms. On March 25, 2011, the U.S. Food and Drug Administration (FDA) approved intravenous Ipilimumab® at a dosage of 3 mg/kg as treatment for metastatic melanoma, regardless of prior therapy [13, 31, 32]. It was soon recommended in the National Comprehensive Cancer Network (NCCN) guidelines as a systemic therapy option for advanced melanoma [33]. The European Medicines Agency (EMA) approved it in November 2013 as a first-line treatment [34].

Ipilimumab® was the first checkpoint inhibitor approved for melanoma, with a phase III trial demonstrating a median overall survival (OS) of 10.1 months, compared to 6.4 months in the control group. Furthermore, durable responses exceeding 5 years were observed in some patients, suggesting the potential for long-term disease control [35].

3.2 PD-1/PD-L1 inhibition: Nivolumab® and Pembrolizumab®

Following CTLA-4 inhibitors, PD-1 and PD-L1 inhibitors were developed. These agents—PD-1 inhibitors like Pembrolizumab® and Nivolumab® and PD-L1 inhibitors such as Atezolizumab®—work by blocking the PD-1/PD-L1 pathway, thereby keeping T-cells active and amplifying their cytotoxic effects [36]. Clinical trials have demonstrated that these inhibitors improve prognosis in advanced melanoma compared to chemotherapy or radiation. However, tumor resistance to these agents is not uncommon [36, 37].

3.2.1 Mechanism of action

PD-1 inhibitors represent a novel strategy in solid tumor immunotherapy. Landmark trials like KEYNOTE-006 and CheckMate-067 have shown impressive response rates in metastatic melanoma, significantly improving both progression-free and overall survival [36, 38, 39]. Pembrolizumab® and Nivolumab® enhance the immune system's tumor-specific response by restoring its ability to recognize and eliminate malignant cells [40].

PD-1 is expressed only on activated T-cells and is absent in resting ones [41–43]. In chronic infections or cancer, persistent stimulation leads to high PD-1 expression, causing T-cell exhaustion and reduced effector function [41].

PD-1 inhibitors block the interaction between PD-1 on T-cells and its ligands (PD-L1/PD-L2), which are often overexpressed by melanoma cells. This blockade restores T-cell activity, enabling CTLs to efficiently recognize and lyse melanoma cells. PD-L1 inhibitors, such as atezolizumab, block the ligand on tumors and APCs, disrupting immune evasion and enhancing T-cell function [40, 41, 44–46].

Blocking PD-1 also prevents inhibition of ZAP-70 and PI3K/AKT signaling, which are vital for T-cell survival. This leads to elevated Tumor Necrosis Factor-alpha (TNF- α) and Interferon-gamma (IFN- γ) in the TME, amplifying the immune response [40, 47]. Moreover, PD-1 inhibitors increase chemokine secretion (CXCL9 and CXCL10), attracting naïve T-cells and boosting antigen presentation [40, 48].

While PD-1 inhibitors target T-cell receptors, PD-L1 inhibitors act on tumor- or APC-expressed ligands—resulting in broader immune activation [40]. The increased cytotoxicity leads to melanoma cell lysis *via* perforins and granzymes, contributing to durable tumor control. Both inhibitor types also transform the TME from immunosuppressive to immunogenic [40, 49].

3.2.2 Regulatory approvals

Nivolumab® and Pembrolizumab® are the only FDA- and EMA-approved anti-PD-1 antibodies for melanoma.

Nivolumab® received FDA approval in December 2014 for unresectable/metastatic melanoma after prior Ipilimumab® and BRAF inhibitors (for BRAF V600-mutated tumors). In 2015, it was approved by the EMA for advanced melanoma regardless of BRAF status. Its indication was later expanded to adjuvant treatment after lymph node involvement and complete resection.

Pembrolizumab® gained FDA approval in September 2014 for metastatic melanoma after ipilimumab, followed by EMA approval in 2015. It is approved for both first-line and adjuvant settings.

3.2.3 Clinical evidence

Numerous trials support the safety and efficacy of PD-1 inhibitors in advanced and resected stage III melanoma [50, 51].

- *KEYNOTE-001 (Phase Ib)*: Five-year OS was 34% in treatment-naïve patients vs. 23% in pretreated ones. Median survival: 38.6 vs. 23.8 months [51].
- *KEYNOTE-006*: Pembrolizumab® vs. Ipilimumab®—12-month OS: 74.1% vs. 58.2%; ORR: 33.7% vs. 11.9% [50].
- *KEYNOTE-054*: Pembrolizumab® in high-risk stage III melanoma (adjuvant)—RFS: 50% vs. 36%; DMFS also improved [52].

For Nivolumab®:

- *CheckMate 037*: Post-ipilimumab or BRAF inhibitors—ORR: 31.7% vs. 10.6% (chemo); median OS: 16 vs. 14 months [53].
- *CheckMate 066*: BRAF wild-type, treatment-naïve—5-year OS: 39% (nivolumab) vs. 17% (dacarbazine); Progression-free survival (PFS): 28% vs. 3%. Twenty percent had complete responses; 88% remained alive at 5 years [54].

Combination Therapy Example:

- *IMspire150 trial*: Atezolizumab® + Vemurafenib® + Cobimetinib® improved median PFS (15.1 vs. 10.6 months). Only effective in BRAF V600-mutated melanoma [55].

Monotherapy with PD-L1 inhibitors, such as Atezolizumab®, is approved in combination regimens for BRAF V600-mutated melanoma (e.g., with Cobimetinib® and Vemurafenib®). The IMspire150 trial showed PFS of 15.1 vs. 10.6 months with the combination vs. control [56]. PD-L1 monotherapy showed a 36% median OS and a 45% disease control rate, especially in high PD-L1 expressers [55].

3.2.4 Neoadjuvant use

A pooled analysis of six trials with 573 patients evaluated presurgical use of checkpoint inhibitors. Combination (PD-1 + CTLA-4) showed a stronger pathological response, but PD-1 monotherapy had better safety. Further trials are ongoing to assess benefits in resectable high-risk melanoma [57, 58].

3.2.5 “Cold tumors”

Low-immunogenicity tumors with minimal TIL, known as “cold” tumors, respond poorly to PD-1 inhibitors due to a lack of effector CD8+ T-cells and an immunosuppressive microenvironment [59, 60]. Dense stroma, abundant Tregs, and suppressive cytokines (e.g., TGF-β) inhibit immune activity. Hence, PD-1/PD-L1 inhibitors are generally ineffective and contraindicated in these cases.

Current research aims to convert “cold” tumors into “hot” by combining PD-L1 inhibitors with cancer vaccines, though evidence remains insufficient for clinical application [55, 61, 62].

3.3 Combination therapy: CTLA-4 and PD-1 inhibition

CTLA-4 and PD-1 regulate immune responses at different stages: CTLA-4 acts early in lymph nodes during T-cell priming, while PD-1 suppresses T-cells later in peripheral tissues. Their combined blockade may produce additive or synergistic effects by enhancing both T-cell activation and effector function [63].

While monotherapy with either agent yields durable responses with manageable toxicity, a large subset of patients remains unresponsive. The CheckMate 067 trial demonstrated that nivolumab + ipilimumab offers significantly greater efficacy in metastatic melanoma and was approved for use in several advanced cancers.

Five-year OS rates from CheckMate 067 were 52% (combination), 44% (Nivolumab®), and 26% (Ipilimumab®). Median PFS: 11.5, 6.9, and 2.9 months, respectively [63, 64].

However, combination therapy comes with increased toxicity:

- Severe (Grade 3–4) immune-related adverse events (irAEs) occur in 38–55% of patients [35, 65–71].
- These can lead to dose reductions or treatment discontinuation.
- Rare but serious irAEs include neurological events (encephalitis, neuropathy) and myocarditis [72, 73].

4. Immune-related adverse events (irAEs)

Although immunotherapy is generally considered safe and effective, it is associated with a relatively high incidence of adverse events, mainly due to the broad activation of the immune system. For Ipilimumab®, these adverse events are often mild or moderate but occur in a high percentage of patients (70–88%) and are dose-dependent [37, 74, 75]. Generally, PD-1 inhibitors are considered better tolerated than CTLA-4 inhibitors. However, both categories of immunotherapy carry a significant risk of irAEs due to immune activation.

The most common treatment-related adverse events during infusion or within 24–72 hours after infusion include nausea, vomiting, diarrhea, headache, dizziness, fever, fatigue, and skin conditions such as rash and pruritus. These are usually mild and require only symptomatic treatment, temporary discontinuation, or slowing of the ipilimumab infusion. Tachycardia and hypotension have also been rarely observed around the time of infusion [76]. Severe (Grade 3–4) adverse events may occur in approximately 27% of patients receiving ipilimumab as monotherapy [77]. These toxicities often necessitate treatment interruption, corticosteroid administration, or immunosuppressive therapy.

IrAEs may present at any point during the treatment process, but they commonly occur during the third or fourth dose of ipilimumab. Assessing the severity of irAEs using toxicity grading criteria is essential; treatment may include steroids and, if

needed, discontinuation of further immunotherapy. In some cases, severe irAEs (grade 3/4) can be life-threatening [78]. However, they are not very common (5–25% for Ipilimumab® and 10–15% for PD-1/PD-L1 inhibitors) and are mainly dose-related [37, 62, 74, 75, 77, 79–81].

The most common irAEs in melanoma immunotherapy may affect various organs and systems. Cutaneous adverse events may include dermatitis, rash, or pruritus. These are common in PD-1/PD-L1 monotherapy, affecting approximately 20–30% of patients. The endocrine system is probably the most critical among the common irAEs, as endocrinopathies of various types and severity may occur. These include thyroiditis and hypophysitis for CTLA-4 inhibitors, while hypothyroidism, type 1 diabetes, or adrenal insufficiency are more common among PD-1/PD-L1 inhibitors. Gastrointestinal tract adverse events may include diarrhea, enterocolitis, and liver abnormalities such as hepatitis and elevated liver enzymes. Other irAEs clinicians should be aware of, though less common, include uveitis and pneumonitis. Less frequent irAEs may also affect the nervous system (central or peripheral deficits), as well as the hematopoietic and cardiovascular systems.

Common irAEs affect multiple organ systems [76, 77, 80–83]:

- Skin: Rash, pruritus, and dermatitis occur in 20–30% of patients.
- Endocrine system: Endocrinopathies such as thyroiditis and hypophysitis (more common with CTLA-4 inhibitors), hypothyroidism, adrenal insufficiency, and type 1 diabetes (more often with PD-1/PD-L1 inhibitors).
- Gastrointestinal tract: Diarrhea, colitis, and immune-mediated hepatitis with elevated liver enzymes.
- Other: Uveitis, pneumonitis, and rare but severe neurologic (e.g., encephalitis, neuropathy), cardiovascular, and hematopoietic adverse events.

These adverse events mimic autoimmune conditions (e.g., autoimmune hepatitis, inflammatory bowel disease), making them difficult to distinguish. However, the pathophysiology of ipilimumab-induced irAEs is distinct from classical autoimmune conditions and requires appropriate management and treatment strategies [68, 76, 84]. Primary treatment is supportive care, while more severe irAEs require high-dose steroids and cessation of Ipilimumab®. However, permanent discontinuation of immunotherapy is typically not necessary. Some studies have shown a correlation between the development of irAEs and improved antitumor response [75, 76, 85, 86]. In some cases, antitumor activity continues despite steroid therapy for irAEs [86–88]. However, efficacy may be compromised; therefore, steroids should be initiated when necessary but avoided if not essential [76].

5. Biomarkers and risk prediction

Personalized medicine, particularly in immunotherapy and cancer treatment strategies, represents the future of oncology. Emerging evidence suggests that biomarkers will soon become standard tools in clinical practice, with more currently under investigation, aiming to ensure that patients receive optimal therapy for their malignancy while minimizing the risk of severe irAEs [89].

There are currently three main categories of predictive biomarkers for melanoma immunotherapy: tumor-related factors, host-related factors, and the TME. These biomarkers include a variety of components, but the most important under investigation include gene mutations, immune cell signatures, PD-L1 expression, toll-like receptor signaling, TNF α signaling, and microRNA biogenesis [89–91].

Three primary biomarker categories under investigation are as follows:

- Tumor-related factors (e.g., genetic mutations),
- Host-related factors (e.g., immune profiles), and
- TME characteristics (e.g., PD-L1 expression, immune infiltration).

Future prediction models may incorporate multiple biomarkers to assess both prognosis and irAE risk in a multifactorial manner [89, 91]. Ongoing studies are developing models capable of identifying antibody signatures that may effectively stratify patients based on toxicity profiles, with reported sensitivity, accuracy, and specificity above 90% [89].

6. Other novel types of immunotherapy treatment

6.1 T-VEC immunotherapy

Talimogene laherparepvec (T-VEC) immunotherapy is used in advanced (unresectable) and metastatic melanoma *via* some intralesional injections. It uses a modified oncolytic herpes virus, and it is the first intralesional therapy using an oncolytic virus that has received approval from both the FDA and EMA [40, 92]. Its use as a monotherapy has shown some promising results; a recent multicenter review reported the potential for T-VEC to be used as a salvage pathway for those patients who have limited treatment options, especially after an ineffective immunotherapy treatment course [40, 93]. However, being a novel oncolytic immunotherapy, cost is a burden for patient access to the treatment, while handling, storage, and transportation are also very demanding, limiting the availability among providers [94, 95]. Furthermore, there is always a consideration that the virus used for the treatment may rapidly mutate and be pathogenic. For this reason, it should not be used without consideration for patients on high-dose immunosuppressants or those suffering from autoimmune disease [96]. In summary, T-VEC seems to be a significant innovation in cancer immunotherapy, yet there are still significant challenges in terms of logistics and production that limit its widespread availability for patients.

6.2 mRNA vaccine

mRNA vaccines have recently been developed and put into clinical testing and practice for various conditions, including melanoma treatment. Their mechanism of action is based on encoding tumor-specific neoantigens, promoting cellular uptake and neoantigen presentation, and amplifying the adaptive immunity of the treated patient. The unique mutations of a patient's melanoma allowed the researchers to develop the new mRNA vaccines by encoding tumor-specific neoantigens. When exposing the immune system of the patient to these neoantigens, it results in precision

targeting of the malignant cells, while healthy tissue stays intact, thus enforcing a tumor-specific immune response [97].

mRNA vaccines use lipid nanoparticles (LNPs) to deliver the treatment through mRNA encapsulation so that it can be protected from proteases' action and be safely delivered to APCs *via* endocytosis. Subsequently, the patient's immune system gets activated, leveraging both acute and memory immune responses, resulting in efficacy and targeted management of the tumor. The Phase 2b KEYNOTE-942 trial on mRNA-4157/V940, combined with Pembrolizumab®, showed better results than traditional chemotherapy or radiation [98].

Although the overall idea of personalized mRNA vaccines is promising, there are still challenges that scientists need to overcome. The need for specialized scientists for its development, the high cost of equipment, and the time-consuming process for its development are currently burdens for its application in clinical settings. Furthermore, there are still regulatory requirements from the FDA that need to be addressed concerning the safety and development of the mRNA therapies [99–101].

6.2.1 mRNA vaccine + PD-1 inhibitors

The combination of mRNA vaccines and PD-1 inhibitors has already shown some promising results in better melanoma outcomes [32]. The KEYNOTE-942 trial, a phase IIb open-label randomized clinical trial, assessed the efficacy of Pembrolizumab® in combination with the individualized neoantigen mRNA-4157 (V940) vaccine compared to Pembrolizumab® monotherapy in patients with full resection of high-risk melanoma [98]. The study reported better results, with longer recurrence-free survival and metastasis-free survival in the combination therapy group versus Pembrolizumab® monotherapy, supported by a lower recurrence and death rate, as well as a higher 18-month recurrence-free survival. Furthermore, the safety profile was comparable to monotherapy, with adverse events reported in approximately 36% of patients in both groups. The authors suggested that mRNA-4157 neoantigen therapy enhanced the anti-PD-1 immunotherapy by increasing both endogenous and de novo T-cell responses [40, 98]. Another phase I study, KEYNOTE-603, investigated the same individualized neoantigen therapy in combination with pembrolizumab or as monotherapy among patients with completely resected stage II–IV melanoma. It resulted in similar results as the KEYNOTE-942 trial, with enhanced clinical benefits and safety profiles, as shown in KEYNOTE-603. Furthermore, it evaluated the immunogenicity, reporting neoantigen-specific T-cell responses that elicited the release of TNF- α and IFN- γ [102].

6.3 Tumor-infiltrating lymphocyte (TIL) therapy

TIL therapy involves an adoptive cell transfer (ACT) of the patient's cells, specifically T-cells, into their tumor. The main goal is to increase, in an artificial way, the number of tumor-specific T-cells required to control tumor cell proliferation. These are already differentiated and specialized T-cells that target melanoma antigens. These T-cells are often ineffective due to the high levels of immune checkpoint receptor activity. Investigators have conducted trials examining the clinical impact of the CTLA-4 blockade [103–105]. TIL therapy is enhanced by the use of the CTLA-4 inhibitor Ipilimumab®, due to the prevention of T-cell suppression. However, significantly higher immune-related adverse events have been reported [104, 105].

6.3.1 TIL therapy + PD-1 inhibitors

TIL therapy in combination with anti-PD-1 inhibitors has shown promising results in melanoma treatment. Nivolumab in combination with ACT using TILs expanded with anti-4-1BB agonism has been clinically tested among stage III/IV metastatic melanoma treatment-naïve patients. The median progression-free survival was estimated at 5 months, but two out of 11 patients did not respond to therapy and developed new metastatic lesions. Clonal divergence and intrinsic TIL dysfunction are the suspected mechanisms of therapeutic resistance [106, 107]. Another phase I/II study investigated a treatment course of an initial anti-PD-1 therapy, followed by two subsequent injections of TILs, and finally a second round of anti-PD-1 therapy, showing some promising results [108]. Thus, the TIL therapy and PD-1 inhibitor combination are a potential treatment for metastatic melanoma, but further research is needed in order to come to safe conclusions. However, despite the promising outcomes that were reported, it seems that prior exposure to anti-PD-1 therapies may reduce the effectiveness of the TIL therapy [109, 110].

6.3.2 TIL therapy + mRNA vaccines

Although the combination of TIL therapy and mRNA vaccines represents an intriguing area of research, there are still no clinical trials that have investigated their synergistic potential. Future studies need to explore this combination that may provide insights into these novel immunotherapeutic strategies for melanoma [40].

7. Challenges and limitations

Although the efficacy of immunotherapy in melanoma is established by evidence, there are still challenges and limitations concerning its clinical implementation. Resistance is one of the main challenges, divided into two main forms: the primary and the acquired. Primary resistance is characterized by the inherent lack of ability to respond to PD-1 and PD-L1 inhibitors because of the TME caused by insufficient PD-1/PD-L1 expression, lower TMB, and disruption of the critical immune signaling pathways. On the other hand, acquired resistance is characterized by resistance developed by adaptive mutations caused by the tumor cells [111, 112].

Another limitation is the cost of the immunotherapy and the limited availability worldwide, especially in countries that do not have the means to offer this treatment option to all the patients for free or at low cost. Patients who may not have this treatment covered by their insurance or country expenses are usually unable to support such a high cost on their own. Toxicity and adverse events are common and even life-threatening in some cases. Thus, the selection of patients to receive immunotherapy should be careful and based on various factors and criteria. Finally, there are specific populations that are underrepresented in clinical trials; thus, the efficacy and safety are not tested as they should be. These populations include elderly people, who are commonly affected by melanoma, as well as some ethnic groups.

8. Conclusion

Immunotherapy has fundamentally reshaped the therapeutic landscape of melanoma. It has transformed the way we manage a disease that was once a highly lethal

malignancy into one that can be effectively controlled these days or even durably suppressed in some cases. The introduction of ICIs (CTLA-4 and PD-1/PD-L1) has extended survival and has offered long-term remission to a high percentage of patients with advanced disease. Durable responses are now achievable both with monotherapy and combination treatment, while there are novel emerging strategies that continue to expand and refine these benefits.

Beyond the survival statistics, the impact of immunotherapy has affected the quality of life of these patients, offering sustained disease control and manageable side effects, unlike cytotoxic chemotherapy. The ability of these patients to return to work, regain physical function, and engage in family and social life activities has become a reality for individuals who have previously faced a terminal prognosis of melanoma.

The complexity of melanoma treatment requires a multidisciplinary approach that demands the expertise of various specialties, including oncologists, pathologists, dermatologists, surgeons, radiologists, and supportive care teams or other specialties based on adverse effects that may occur. Early diagnosis of the adverse events, coordinated long-term follow-up, and optimal patient selection are essential to maximize the therapeutic success and to eliminate toxicity. Multidisciplinary collaboration ensures that decisions for the treatment and follow-up are patient-centered and up-to-date with current evidence.

Looking forward, continued research and innovation are essential for the future. While checkpoint inhibitors have set a new benchmark in melanoma treatment, there are still numerous challenges, including resistance, toxicities, and limited efficacy in specific melanoma subtypes. The research on novel immune targets, personalized immunotherapies, and biomarkers for assessment of response and toxicity, along with combination regimens with already tested and safe systemic or locoregional therapies, must remain a priority in scientific society. Finally, global access to immunotherapy should be a matter of high importance, as the advances in melanoma care should not just be limited to well-resourced healthcare systems but also to the rest of the world.

In conclusion, immunotherapy is one of the most significant advances in melanoma treatment in the last couple of decades. Its integration into clinical practice has been a breakthrough, extending lives and improving quality of care and long-term outcomes. As we continue to build on this progress, sustained investment in global health equity, science, and collaborative care is essential to fully realize the potential that these technological and pharmacological advancements have to offer to humanity.

Author details

Areti Katsouda^{1*}, Thomas Rallis², Apostolos Gogakos², Vasileios Theocharidis², Thomas Kanteres², Myrtw Tzinevi², Ioannis Passos³ and Maria Mironidou-Tzouveleki⁴

1 Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

2 Thoracic Surgery Department, “Theageneio” Cancer Hospital, Thessaloniki, Greece

3 Surgical Department, 424 Army Hospital, Thessaloniki, Greece

4 Laboratory of Pharmacology, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

*Address all correspondence to: katsoudaareti@gmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Arnold M, Singh D, Laversanne M, Vignat J, Vaccarella S, Meheus F, et al. Global burden of cutaneous melanoma in 2020 and projections to 2040. *JAMA Dermatology*. 2022;**158**:495. DOI: 10.1001/JAMADERMATOL.2022.0160
- [2] De Pinto G, Mignozzi S, La Vecchia C, Levi F, Negri E, Santucci C. Global trends in cutaneous malignant melanoma incidence and mortality. *Melanoma Research*. 2024;**34**:265-275. DOI: 10.1097/CMR.0000000000000959
- [3] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*. 2021;**71**:209-249. DOI: 10.3322/CAAC.21660
- [4] Del Marmol V. Prevention and screening of melanoma in Europe: 20 years of the Euromelanoma campaign. *Journal of the European Academy of Dermatology and Venereology*. 2022;**36**:5-11. DOI: 10.1111/JDV.18195
- [5] Rigel DS, Russak J, Friedman R. The evolution of melanoma diagnosis: 25 years beyond the ABCDs. *CA: A Cancer Journal for Clinicians*. 2010;**60**:301-316. DOI: 10.3322/CAAC.20074
- [6] Rashid S, Shaughnessy M, Tsao H. Melanoma classification and management in the era of molecular medicine. *Dermatologic Clinics*. 2023;**41**:49-63. DOI: 10.1016/J.DET.2022.07.017
- [7] Heistein JB, Acharya U, Mukkamalla SKR. *Malignant Melanoma*. Treasure Island, Florida, USA: StatPearls; 2024
- [8] Eggermont AMM, Kirkwood JM. Re-evaluating the role of dacarbazine in metastatic melanoma: What have we learned in 30 years? *European Journal of Cancer*. 2004;**40**:1825-1836. DOI: 10.1016/J.EJCA.2004.04.030
- [9] Kim C, Lee CW, Kovacic L, Shah A, Klasa R, Savage KJ. Long-term survival in patients with metastatic melanoma treated with DTIC or Temozolomide. *The Oncologist*. 2010;**15**:765. DOI: 10.1634/THEONCOLOGIST.2009-0237
- [10] Serrone L, Zeuli M, Segal FM, Cognetti F. Dacarbazine-based chemotherapy for metastatic melanoma: Thirty-year experience overview. *Journal of Experimental & Clinical Cancer Research*. 2000;**19**:21-34
- [11] Knight A, Karapetyan L, Kirkwood JM. Immunotherapy in melanoma: Recent advances and future directions. *Cancers (Basel)*. 2023;**15**. DOI: 10.3390/CANCERS15041106
- [12] Lugowska I, Teterycz P, Rutkowski P. Immunotherapy of melanoma. *Contemporary Oncology/Współczesna Onkologia*. 2018;**2018**:61-67. DOI: 10.5114/WO.2018.73889
- [13] Savoia P, Astrua C, Fava P. Ipilimumab (Anti-Ctla-4 Mab) in the treatment of metastatic melanoma: Effectiveness and toxicity management. *Human Vaccines & Immunotherapeutics*. 2016;**12**:1092. DOI: 10.1080/21645515.2015.1129478
- [14] Gurjao C, Tsukrov D, Imakaev M, Luquette LJ, Mirny LA. Is tumor mutational burden predictive of response to immunotherapy? *eLife*. 2023;**12**. DOI: 10.7554/ELIFE.87465.1

- [15] Xie N, Shen G, Gao W, Huang Z, Huang C, Fu L. Neoantigens: Promising targets for cancer therapy. *Signal Transduction and Targeted Therapy*. 2022;**8**:1 2023;**8**:1-38. DOI: 10.1038/s41392-022-01270-x
- [16] Alban TJ, Riaz N, Parthasarathy P, Makarov V, Kendall S, Yoo S-K, et al. Neoantigen immunogenicity landscapes and evolution of tumor ecosystems during immunotherapy with nivolumab. *Nature Medicine*. 2024;**30**:3209. DOI: 10.1038/S41591-024-03240-Y
- [17] Szczepaniak Sloane RA, Gopalakrishnan V, Reddy SM, Zhang X, Reuben A, Wargo JA. Interaction of molecular alterations with immune response in melanoma. *Cancer*. 2017;**123**:2130-2142. DOI: 10.1002/CNC R.30681;REQUESTEDJOURNAL:JOURNAL:10970142;WGROU:STRING:PUBLICATION
- [18] Georgoulis G, Zaravinos A. Genomic landscape of the immunogenicity regulation in skin melanomas with diverse tumor mutation burden. *Frontiers in Immunology*. 2022;**13**:1006665. DOI: 10.3389/FIMMU.2022.1006665/XML/NLM
- [19] Ko JS. The immunology of melanoma. *Clinics in Laboratory Medicine*. 2017;**37**:449-471. DOI: 10.1016/J.CLL.2017.06.001
- [20] Linette GP, Becker-Hapak M, Skidmore ZL, Baroja ML, Xu C, Hundal J, et al. Immunological ignorance is an enabling feature of the oligo-clonal T cell response to melanoma neoantigens. *Proceedings of the National Academy of Sciences*. 2019;**116**:23662-23670. DOI: 10.1073/PNAS.1906026116
- [21] Inozume T, Fukushima S. Clues for developing next-generation cancer immunotherapy. *Experimental Dermatology*. 2023;**32**:238-239. DOI: 10.1111/EXD.14751;WGROU:STRING:PUBLICATION
- [22] Bai X, Quek C. Unravelling tumour microenvironment in melanoma at single-cell level and challenges to checkpoint immunotherapy. *Genes*. 2022;**13**:1757. DOI: 10.3390/GENES13101757
- [23] Killock D. Paradoxical roles of mutational load as a determinant of anticancer immunity. *Nature Reviews. Clinical Oncology*. 2019;**16**:659-659. DOI: 10.1038/S41571-019-0282-5
- [24] Kun IZ, Kun I, Hadnagy M, Kolcsár M. Therapeutic importance, general and endocrine adverse effects of immune checkpoint inhibitors I: Mechanism of action and therapeutic use. *Bulletin of Medical Sciences*. 2022;**95**:145-166. DOI: 10.2478/ORVTUDERT-2022-0010
- [25] Hoos A, Ibrahim R, Korman A, Abdallah K, Berman D, Shahabi V, et al. Development of ipilimumab: Contribution to a new paradigm for cancer immunotherapy. *Seminars in Oncology*. 2010;**37**:533-546. DOI: 10.1053/j.seminoncol.2010.09.015
- [26] Lipson EJ, Drake CG. Ipilimumab: An anti-CTLA-4 antibody for metastatic melanoma. *Clinical Cancer Research*. 2011;**17**:6958-6962. DOI: 10.1158/1078-0432.CCR-11-1595
- [27] Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, et al. A new member of the immunoglobulin superfamily-CTLA-4. *Nature*. 1988;**328**:267-270. DOI: 10.1038/328267A0
- [28] Brunet J-F, Denizot F, Golstein P. A differential molecular biology search for genes preferentially expressed

in functional T lymphocytes: The CTLA genes. *Immunological Reviews*. 1988;**103**:21-36. DOI: 10.1111/J.1600-065X.1988.TB00747.X

[29] Peggs KS, Quezada SA, Chambers CA, Korman AJ, Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. *Journal of Experimental Medicine*. 2009;**206**:1717-1725. DOI: 10.1084/JEM.20082492

[30] Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *Journal of Experimental Medicine*. 1995;**182**:459-465. DOI: 10.1084/JEM.182.2.459

[31] Squibb BM. FDA Approves YERVOY™ (Ipilimumab) for the Treatment of Patients with Newly Diagnosed or Previously-Treated Unresectable or Metastatic Melanoma, the Deadliest Form of Skin Cancer. 2011

[32] Drug Approval Package: YERVOY (Ipilimumab) n.d. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125377Orig1s000TOC.cfm [Accessed: October 14, 2020]

[33] Coit DG, Andtbacka R, Anker CJ, Bichakjian CK, Carson WE, Daud A, et al. Melanoma, version 2.2013: Featured updates to the NCCN guidelines. *Journal of the National Comprehensive Cancer Network*. 2013;**11**:395-407. DOI: 10.6004/JNCCN.2013.0055

[34] Yervoy|European Medicines Agency (EMA). n.d. Available from: <https://www.ema.europa.eu/en/medicines/human/EPAR/yervoy> [Accessed: June 11, 2025]

[35] Madden KM, Hoffner B. Ipilimumab-based therapy: Consensus statement from

the faculty of the melanoma nursing initiative on managing adverse events with ipilimumab monotherapy and combination therapy with nivolumab. *Clinical Journal of Oncology Nursing*. 2017;**21**:30-41. DOI: 10.1188/17.CJON.S4.30-41

[36] Shah V, Panchal V, Shah A, Vyas B, Agrawal S, Bharadwaj S. Immune checkpoint inhibitors in metastatic melanoma therapy (review). *Medicine International*. 2024;**4**. DOI: 10.3892/MI.2024.137

[37] Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *The New England Journal of Medicine*. 2010;**363**:711. DOI: 10.1056/NEJMOA1003466

[38] Larkin J, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Lao CD, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. *New England Journal of Medicine*. 2019;**381**:1535-1546. DOI: 10.1056/NEJMOA1910836/SUPPL_FILE/NEJMOA1910836_DATA-SHARING.PDF

[39] Robert C, Carlino MS, McNeil C, Ribas A, Grob JJ, Schachter J, et al. Seven-year follow-up of the phase III KEYNOTE-006 study: Pembrolizumab versus ipilimumab in advanced melanoma. *Journal of Clinical Oncology*. 2023;**41**:3998-4003. DOI: 10.1200/JCO.22.01599

[40] Mehta A, Motavaf M, Nebo I, Luyten S, Osei-Opare KD, Gru AA. Advancements in melanoma treatment: A review of PD-1 inhibitors, T-VEC, mRNA vaccines, and tumor-infiltrating lymphocyte therapy in an evolving landscape of immunotherapy. *Journal of Clinical Medicine*. 2025;**14**:1200. DOI: 10.3390/JCM14041200

- [41] Rausch MP, Hastings KT. Immune checkpoint inhibitors in the treatment of melanoma: From basic science to clinical application. In: *Cutaneous Melanoma: Etiology and Therapy*. 2017. pp. 121-142. DOI: 10.15586/CODON.CUTANEOUSMELANOMA.2017.CH9
- [42] Nishimura H, Agata Y, Kawasaki A, Sato M, Imamura S, Minato N, et al. Developmentally regulated expression of the PD-1 protein on the surface of double-negative (CD4-CD8-) thymocytes. *International Immunology*. 1996;**8**:773-780. DOI: 10.1093/INTIMM/8.5.773
- [43] Chemnitz JM, Parry RV, Nichols KE, June CH, Riley JL. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *The Journal of Immunology*. 2004;**173**:945-954. DOI: 10.4049/JIMMUNOL.173.2.945
- [44] Oliveira LJC, Gongora ABL, Jardim DLF. Spectrum and clinical activity of PD-1/PD-L1 inhibitors: Regulatory approval and under development. *Current Oncology Reports*. 2020;**22**. DOI: 10.1007/S11912-020-00928-5
- [45] Surmiak E, Magiera-Mularz K, Musielak B, Muszak D, Kocik-Krol J, Kitel R, et al. PD-L1 inhibitors: Different classes, activities, and mechanisms of action. *International Journal of Molecular Sciences*. 2021;**22**. DOI: 10.3390/IJMS222111797
- [46] Kakavand H, Rawson RV, Pupo GM, Yang JYH, Menzies AM, Carlino MS, et al. PD-L1 expression and immune escape in melanoma resistance to MAPK inhibitors. *Clinical Cancer Research*. 2017;**23**:6054-6061. DOI: 10.1158/1078-0432.CCR-16-1688/128970/AM/PD-L1-EXPRESSION-AND-IMMUNE-ESCAPE-IN-MELANOMA
- [47] Peng W, Liu C, Xu C, Lou Y, Chen J, Yang Y, et al. PD-1 blockade enhances T-cell migration to tumors by elevating IFN- γ inducible chemokines. *Cancer Research*. 2012;**72**:5209-5218. DOI: 10.1158/0008-5472.CAN-12-1187/650582/AM/PD-1-BLOCKADE-ENHANCES-T-CELL-MIGRATION-TO-TUMORS
- [48] Chow MT, Ozga AJ, Servis RL, Frederick DT, Lo JA, Fisher DE, et al. Intratumoral activity of the CXCR3 chemokine system is required for the efficacy of anti-PD-1 therapy. *Immunity*. 2019;**50**:1498-1512.e5. DOI: 10.1016/J.IMMUNI.2019.04.010
- [49] Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJM, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*. 2014;**515**:568-571. DOI: 10.1038/NATURE13954
- [50] Long GV, Carlino MS, McNeil C, Ribas A, Gaudy-Marqueste C, Schachter J, et al. Pembrolizumab versus ipilimumab for advanced melanoma: 10-year follow-up of the phase III KEYNOTE-006 study. *Annals of Oncology: Official Journal of the European Society for Medical Oncology*; **35**(12):1191-1199. DOI: 10.1016/j.annonc.2024.08.2330
- [51] Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, et al. Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. *Annals of Oncology*. 2019;**30**:582-588. DOI: 10.1093/ANNONC/MDZ011
- [52] Eggermont AM, Kicinski M, Blank CU, Mandala M, Long GV, Atkinson V, et al. Seven-year analysis of

adjuvant pembrolizumab versus placebo in stage III melanoma in the EORTC1325 / KEYNOTE-054 trial. *European Journal of Cancer*. 2024;211. DOI: 10.1016/j.ejca.2024.114327

[53] Larkin J, Minor D, D'Angelo S, Neyns B, Smylie M, Miller WH, et al. Overall survival in patients with advanced melanoma who received nivolumab versus investigator's choice chemotherapy in CheckMate 037: A randomized, controlled, open-label phase III trial. *Journal of Clinical Oncology*. 2018;36:383-390. DOI: 10.1200/JCO.2016.71.8023

[54] Robert C, Long GV, Brady B, Dutriaux C, Di Giacomo AM, Mortier L, et al. Five-year outcomes with nivolumab in patients with wild-type BRAF advanced melanoma. *Journal of Clinical Oncology*. 2020;38:3937-3946. DOI: 10.1200/JCO.20.00995/SUPPL_FILE/PROTOCOL_JCO.20.00995.PDF

[55] Hamid O, Molinero L, Bolen CR, Sosman JA, Muñoz-Couselo E, Kluger HM, et al. Safety, clinical activity, and biological correlates of response in patients with metastatic melanoma: Results from a phase I trial of atezolizumab. *Clinical Cancer Research*. 2019;25:6061-6072. DOI: 10.1158/1078-0432.CCR-18-3488/2120687/1078-0432_CCR-18-3488V1.PDF

[56] Robert C, Lewis KD, Gutzmer R, Strydom D, Gogas H, Protsenko S, et al. Biomarkers of treatment benefit with atezolizumab plus vemurafenib plus cobimetinib in BRAFV600 mutation-positive melanoma. *Annals of Oncology*. 2022;33:544-555. DOI: 10.1016/j.annonc.2022.01.076

[57] Mangla A, Lee C, Mirsky MM, Wang M, Rothermel LD, Hoehn R, et al. Neoadjuvant dual checkpoint inhibitors vs anti-PD1 therapy in high-risk

resectable melanoma: A pooled analysis. *JAMA Oncology*. 2024;10:612-620. DOI: 10.1001/JAMAONCOL.2023.7333

[58] Wu D, Huang H, Zhang M, Li Z, Wang S, Yu Y, et al. The global landscape of neoadjuvant and adjuvant anti-PD-1/PD-L1 clinical trials. *Journal of Hematology & Oncology*. 2022;15. DOI: 10.1186/S13045-022-01227-1

[59] Bonaventura P, Shekarian T, Alcazer V, Valladeau-Guilemond J, Valsesia-Wittmann S, Amigorena S, et al. Cold tumors: A therapeutic challenge for immunotherapy. *Frontiers in Immunology*. 2019;10. DOI: 10.3389/FIMMU.2019.00168

[60] Majidpoor J, Mortezaee K. The efficacy of PD-1/PD-L1 blockade in cold cancers and future perspectives. *Clinical Immunology*. 2021;226. DOI: 10.1016/J.CLIM.2021.108707

[61] Chen S, Zhang Z, Zheng X, Tao H, Zhang S, Ma J, et al. Response efficacy of PD-1 and PD-L1 inhibitors in clinical trials: A systematic review and meta-analysis. *Frontiers in Oncology*. 2021;11:562315. DOI: 10.3389/FONC.2021.562315/XML/NLM

[62] Ouyang P, Wang L, Wu J, Tian Y, Chen C, Li D, et al. Overcoming cold tumors: A combination strategy of immune checkpoint inhibitors. *Frontiers in Immunology*. 2024;15:1344272. DOI: 10.3389/FIMMU.2024.1344272/XML/NLM

[63] Ramteke AS. Dual immune checkpoints inhibition: Cancer treatment and immunological modes of action. *Journal of Drug Delivery and Therapeutics*. 2023;13:175-187. DOI: 10.22270/JDDTV13I6.5880

[64] Aillón Albán AG, López Naveda FDL, Guzmán Chávez IT,

Arregui Ayala AC, Sivisaca Bonifaz CN. Evaluation of the efficacy of combined immune checkpoint inhibitors in advanced melanoma treatment - Long term outcomes and emerging therapeutic perspectives: A narrative review. *Ibero-American Journal of Health Science Research*. 2024;**4**:153-161. DOI: 10.56183/IBEROJHR.V4I2.663

[65] Schadendorf D, Wolchok JD, Stephen Hodi F, Chiarion-Sileni V, Gonzalez R, Rutkowski P, et al. Efficacy and safety outcomes in patients with advanced melanoma who discontinued treatment with nivolumab and ipilimumab because of adverse events: A pooled analysis of randomized phase II and III trials. *Journal of Clinical Oncology*. 2017;**35**(34):3807-3814. DOI: 10.1200/JCO.2017.73.2289

[66] Maloney A, Giobbie-Hurder A, Fogarasi MC, Ott PA, Hodi FS, Sussman TA, et al. Role of nivolumab maintenance therapy in advanced melanoma patients following severe immune-related adverse events from combination nivolumab and ipilimumab. *Journal of Clinical Oncology*. 2023;**41**:9520-9520. DOI: 10.1200/JCO.2023.41.16_SUPPL.9520

[67] Sznol M, Chen L. Antagonist antibodies to PD-1 and B7-H1 (PD-L1) in the treatment of advanced human cancer. *Clinical Cancer Research*. 2013;**19**:1021-1034. DOI: 10.1158/1078-0432.CCR-12-2063

[68] Rauwerdink D, Frederick DT, Sharova T, Boland GM. Correlation between immune-related adverse events and outcomes in nivolumab/ipilimumab combination therapy for metastatic melanoma. *Journal of Clinical Oncology*. 2020;**38**:58-58. DOI: 10.1200/JCO.2020.38.5_SUPPL.58

[69] Pelster MS, Gruschus SK, Bassett R, Gombos DS, Shephard M, Posada L,

et al. Nivolumab and ipilimumab in metastatic uveal melanoma: Results from a single-arm phase II study. *Journal of Clinical Oncology*. 2021;**39**:599-607. DOI: 10.1200/JCO.20.00605

[70] Mason R, Dearden HC, Nguyen B, Soon JA, Smith JL, Randhawa M, et al. Combined ipilimumab and nivolumab first-line and after BRAF-targeted therapy in advanced melanoma. *Pigment Cell & Melanoma Research*. 2020;**33**:358-365. DOI: 10.1111/PCMR.12831

[71] Pacholczak-Madej R, Grela-Wojewoda A, Puskulluoglu M, Lompart J, Las-Jankowska M, Krawczak K, et al. Early effects of nivolumab and ipilimumab combined immunotherapy in the treatment of metastatic melanoma in Poland: A multicenter experience. *Biomedicine*. 2022;**10**. DOI: 10.3390/BIOMEDICINES10102528

[72] Mehta A, Gupta A, Hannallah F, Koshy T, Reimold S. Myocarditis as an immune-related adverse event with ipilimumab/nivolumab combination therapy for metastatic melanoma. *Melanoma Research*. 2016;**26**(3):319-320. DOI: 10.1097/CMR.0000000000000251

[73] Larkin J, Chmielowski B, Lao CD, Hodi FS, Sharfman W, Weber J, et al. Neurologic serious adverse events associated with nivolumab plus ipilimumab or nivolumab alone in advanced melanoma, including a case series of encephalitis. *The Oncologist*. 2017;**22**(6):709-718. DOI: 10.1634/THEONCOLOGIST.2016-0487

[74] O'Day SJ, Maio M, Chiarion-Sileni V, Gajewski TF, Pehamberger H, Bondarenko IN, et al. Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: A multicenter single-arm phase II study.

Annals of Oncology. 2010;**21**:1712-1717.
DOI: 10.1093/annonc/mdq013

[75] Wolchok JD, Neyns B, Linette G, Negrier S, Lutzky J, Thomas L, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: A randomised, double-blind, multicentre, phase 2, dose-ranging study. *The Lancet Oncology*. 2010;**11**:155-164. DOI: 10.1016/S1470-2045(09)70334-1

[76] Fecher LA, Agarwala SS, Hodi FS, Weber JS. Ipilimumab and its toxicities: A multidisciplinary approach. *The Oncologist*. 2013;**18**:733. DOI: 10.1634/THEONCOLOGIST.2012-0483

[77] Boutros C, Tarhini A, Routier E, Lambotte O, Ladurie FL, Carbonnel F, et al. Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination. *Nature Reviews. Clinical Oncology*. 2016;**13**:473-486. DOI: 10.1038/NRCLINONC.2016.58

[78] Common Terminology Criteria for Adverse Events (CTCAE)[Protocol Development]CTEP. n.d. Available from: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40 [Accessed: June 11, 2025]

[79] Min L, Hodi FS, Giobbie-Hurder A, Ott PA, Luke JJ, Donahue H, et al. Systemic high-dose corticosteroid treatment does not improve the outcome of ipilimumab-related hypophysitis: A retrospective cohort study. *Clinical Cancer Research*. 2015;**21**:749-755. DOI: 10.1158/1078-0432.CCR-14-2353

[80] Blansfield JA, Beck KE, Tran K, Yang JC, Hughes MS, Kammula US, et al. Cytotoxic T-lymphocyte-associated antigen-4 blockade can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. *Journal of Immunotherapy*.

2005;**28**:593. DOI: 10.1097/01.CJI.0000178913.41256.06

[81] Rauwerdink DJW, van Not O, de Meza M, van Doorn R, van der Hage J, van den Eertwegh AJM, et al. Adverse events in anti-PD-1-treated adjuvant and first-line advanced melanoma patients. *Cancers*. 2024;**16**:2656. DOI: 10.3390/CANCERS16152656

[82] Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, et al. Immune-related adverse events with immune checkpoint blockade: A comprehensive review. *European Journal of Cancer*. 2016;**54**:139-148. DOI: 10.1016/J.EJCA.2015.11.016

[83] Delyon J, Mateus C, Lambert T. Hemophilia a induced by ipilimumab. *New England Journal of Medicine*. 2011;**365**:1747-1748. DOI: 10.1056/NEJMC1110923

[84] Min L, Vaidya A, Becker C. Thyroid autoimmunity and ophthalmopathy related to melanoma biological therapy. *European Journal of Endocrinology*. 2011;**164**:303-307. DOI: 10.1530/EJE-10-0833

[85] Almutairi AR, McBride A, Slack M, Erstad BL, Abraham I. Potential immune-related adverse events associated with monotherapy and combination therapy of ipilimumab, nivolumab, and pembrolizumab for advanced melanoma: A systematic review and meta-analysis. *Frontiers in Oncology*. 2020;**10**:507456. DOI: 10.3389/FONC.2020.00091/BIBTEX

[86] Yang JC, Hughes M, Kammula U, Royal R, Sherry RM, Topalian SL, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. *Journal of Immunotherapy*.

2007;**30**:825-830. DOI: 10.1097/CJI.0B013E318156E47E

[87] Harmankaya K, Erasim C, Koelblinger C, Ibrahim R, Hoos A, Pehamberger H, et al. Continuous systemic corticosteroids do not affect the ongoing regression of metastatic melanoma for more than two years following ipilimumab therapy. *Medical Oncology*. 2011;**28**:1140-1144. DOI: 10.1007/S12032-010-9606-0

[88] Downey SG, Klapper JA, Smith FO, Yang JC, Sherry RM, Royal RE, et al. Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. *Clinical Cancer Research*. 2007;**13**:6681-6688. DOI: 10.1158/1078-0432.CCR-07-0187

[89] Weber J, Thompson JA, Hamid O, Minor D, Amin A, Ron I, et al. A randomized, double-blind, placebo-controlled, phase II study comparing the tolerability and efficacy of ipilimumab administered with or without prophylactic budesonide in patients with unresectable stage III or IV melanoma. *Clinical Cancer Research*. 2009;**15**:5591-5598. DOI: 10.1158/1078-0432.CCR-09-1024

[90] Gowen MF, Giles KM, Simpson D, Tchack J, Zhou H, Moran U, et al. Baseline antibody profiles predict toxicity in melanoma patients treated with immune checkpoint inhibitors. *Journal of Translational Medicine*. 2018;**16**:82. DOI: 10.1186/s12967-018-1452-4

[91] Ellebaek E, Khan S, Bastholt L, Schmidt H, Haslund CA, Donia M, et al. PD-L1 is a biomarker of real-world clinical outcomes for anti-CTLA-4 plus anti-PD-1 or anti-PD-1 monotherapy in metastatic melanoma. *European Journal of Cancer*. 2024;**198**:113476. DOI: 10.1016/j.ejca.2023.113476

[92] Ma W, Liu W, Zhong J, Zou Z, Lin X, Sun W, et al. Advances in predictive biomarkers for melanoma immunotherapy. *Holistic Integrative Oncology*. 2024;**3**:1-12. DOI: 10.1007/S44178-024-00121-9

[93] Pol J, Kroemer G, Galluzzi L. First oncolytic virus approved for melanoma immunotherapy. *Oncoimmunology*. 2016;**5**. DOI: 10.1080/2162402X.2015.1115641

[94] Carr MJ, Sun J, DePalo D, Rothermel LD, Song Y, Straker RJ, et al. Talimogene laherparepvec (T-VEC) for the treatment of advanced locoregional melanoma after failure of immunotherapy: An international multi-institutional experience. *Annals of Surgical Oncology*. 2022;**29**:791-801. DOI: 10.1245/S10434-021-10910-5/METRICS

[95] Zhang T, Jou THT, Hsin J, Wang Z, Huang K, Ye J, et al. Talimogene laherparepvec (T-VEC): A review of the recent advances in cancer therapy. *Journal of Clinical Medicine*. 2023;**12**. DOI: 10.3390/JCM12031098

[96] Kalsi S, Galenkamp AL, Singh R, Khosla AA, McGranaghan P, Cintolo-Gonzalez J. Talimogene laherparepvec (T-VEC) and emerging intralesional immunotherapies for metastatic melanoma: A review. *Current Oncology Reports*. 2024;**26**. DOI: 10.1007/S11912-024-01611-9

[97] Sun J, Gastman BR, McCahon L, Buchbinder EI, Puzanov I, Nanni M, et al. Observational study of talimogene laherparepvec use in the anti-PD-1 era for melanoma in the US (COSMUS-2). *Melanoma Management*. 2020;**7**. DOI: 10.2217/MMT-2020-0005

[98] Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P,

- Löwer M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature*. 2017;**547**:7662, 222-6. DOI: 10.1038/nature23003
- [99] Weber JS, Carlino MS, Khattak A, Meniawy T, Ansstas G, Taylor MH, et al. Individualised neoantigen therapy mRNA-4157 (V940) plus pembrolizumab versus pembrolizumab monotherapy in resected melanoma (KEYNOTE-942): A randomised, phase 2b study. *The Lancet*. 2024;**403**:632-644. DOI: 10.1016/S0140-6736(23)02268-7
- [100] Baraniak-Lang I, Amend A-L. The Complex Regulatory Landscape for Personalized Cancer Vaccines. *ACRP*; 2024. Available from: <https://acrpnet.org/2024/10/22/the-complex-regulatory-landscape-for-personalized-cancer-vaccines> [Accessed: June 14, 2025]
- [101] Shemesh CS, Hsu JC, Hosseini I, Shen BQ, Rotte A, Twomey P, et al. Personalized cancer vaccines: Clinical landscape, challenges, and opportunities. *Molecular Therapy*. 2021;**29**:555-570. DOI: 10.1016/J.YMTHE.2020.09.038
- [102] Plotkin S, Robinson JM, Cunningham G, Iqbal R, Larsen S. The complexity and cost of vaccine manufacturing – An overview. *Vaccine*. 2017;**35**:4064-4071. DOI: 10.1016/J.VACCINE.2017.06.003
- [103] Gainor JF, Patel MR, Weber JS, Gutierrez M, Bauman JE, Clarke JM, et al. T-cell responses to individualized neoantigen therapy mRNA-4157 (V940) alone or in combination with pembrolizumab in the phase 1 KEYNOTE-603 study. *Cancer Discovery*. 2024;**14**(11):2209-2223:OF1-15. DOI: 10.1158/2159-8290.CD-24-0158
- [104] Hirai I, Funakoshi T, Kamijuku H, Fukuda K, Mori M, Sakurai M, et al. Adoptive cell therapy using tumor-infiltrating lymphocytes for melanoma refractory to immune-checkpoint inhibitors. *Cancer Science*. 2021;**112**:3163-3172. DOI: 10.1111/CAS.15009
- [105] Besser MJ, Itzhaki O, Zippel D, Shapira-Frommer R, Treves AJ, Zippel DB, et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: Intent-to-treat analysis and efficacy after failure to prior immunotherapies. *Clinical Cancer Research*. 2013;**19**:4792-4800. DOI: 10.1158/1078-0432.CCR-13-0380
- [106] Zhang H, Dai Z, Wu W, Wang Z, Zhang N, Zhang L, et al. Regulatory mechanisms of immune checkpoints PD-L1 and CTLA-4 in cancer. *Journal of Experimental and Clinical Cancer Research*. 2021;**40**. DOI: 10.1186/S13046-021-01987-7
- [107] Hall MS, Mullinax JE, Cox CA, Hall AM, Beatty MS, Blauvelt J, et al. Combination nivolumab, CD137 agonism, and adoptive cell therapy with tumor-infiltrating lymphocytes for patients with metastatic melanoma. *Clinical Cancer Research*. 2022;**28**:5317-5329. DOI: 10.1158/1078-0432.CCR-22-2103
- [108] L’Orphelin JM, Lancien U, Nguyen JM, Coronilla FJS, Saiagh S, Cassecuel J, et al. NIVO-TIL: Combination anti-PD-1 therapy and adoptive T-cell transfer in untreated metastatic melanoma: An exploratory open-label phase I trial. *Acta Oncologica*. 2024;**63**:867-877. DOI: 10.2340/1651-226X.2024.40495
- [109] Seitter SJ, Sherry RM, Yang JC, Robbins PF, Shindorf ML, Copeland AR, et al. Impact of prior treatment on the efficacy of adoptive transfer of

tumor-infiltrating lymphocytes in patients with metastatic melanoma. *Clinical Cancer Research*. 2021;**27**:5289-5298. DOI: 10.1158/1078-0432.CCR-21-1171

[110] Levi ST, Copeland AR, Nah S, Crystal JS, Ivey GD, Lalani A, et al. Neoantigen identification and response to adoptive cell transfer in anti-PD-1 naïve and experienced patients with metastatic melanoma. *Clinical Cancer Research*. 2022;**28**:3042-3052. DOI: 10.1158/1078-0432.CCR-21-4499

[111] Gide TN, Wilmott JS, Scolyer RA, Long GV. Primary and acquired resistance to immune checkpoint inhibitors in metastatic melanoma. *Clinical Cancer Research*. 2018;**24**:1260-1270. DOI: 10.1158/1078-0432.CCR-17-2267

[112] Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell*. 2017;**168**:707-723. DOI: 10.1016/j.cell.2017.01.017

Liver Cancer Immune Microenvironment and Immunotherapy

Chang Liu, Yi-Jun Tu and Li Zhang

Abstract

Liver cancer, especially hepatocellular carcinoma (HCC), is induced by inflammation, which makes HCC a representative target for immune-based approaches. The progression of HCC is regulated by the immune microenvironment. Cytotoxic T lymphocyte protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), lymphocyte activation gene 3 protein (LAG-3), B- and T-lymphocyte attenuator (BTLA), and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) are the most studied immune checkpoints. In HCC, clinical development mostly focused on CTLA-4 and PD-1/programmed cell death ligand 1 (PD-L1) pathways. Immune checkpoint inhibitors (ICIs) against CTLA-4 and PD-1 have revolutionized cancer therapy and have gained increased interest in the treatment of HCC. In this review, we will discuss the liver immune microenvironment and the immunotherapeutic approaches, especially ICIs, against HCC.

Keywords: liver cancer, immunotherapy, immune microenvironment, immune checkpoint inhibitors, combination therapy

1. Introduction

According to the global cancer statistics in 2022, liver cancer is the sixth most prevalent cancer and the third leading cause of cancer-related death in the world, with hepatocellular carcinoma (HCC) comprising 75–85% of cases [1]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for 56% and 20% of liver cancer deaths worldwide, respectively, making HCC an inflammation-related disease [2]. Early-stage HCC is amenable to curative therapies—resection, liver transplantation, or local ablation [3]. At a more developed stage, HCC does not respond to classical chemotherapies [4]. Instead, chemoembolization (for intermediate HCC) and systemic therapies, such as tyrosine kinase inhibitors (TKIs) (for advanced HCC), have shown improvement in patient outcomes [4]. The frontline TKIs, including sorafenib and lenvatinib, target the RAF-mitogen-activated protein kinase (MAPK) kinase (MEK)-extracellular signal-related kinase (ERK) cascade and angiogenesis via vascular endothelial growth factor receptor (VEGFR) [5]. In 2007, the US Food and Drug Administration (FDA) approved the use of sorafenib in advanced-stage HCC [4]. In recent years, three TKIs—lenvatinib, regorafenib, and cabozantinib—have been approved [6]. Nevertheless, compared with

placebo, sorafenib only confers a survival benefit of 2.8 months [7]. Additionally, lenvatinib demonstrated a slightly improved median overall survival compared to sorafenib, with durations of 13.6 months and 12.3 months, respectively [7]. Moreover, regorafenib increased patient survival after progression on sorafenib [8].

Over the past decade, the advent of immunotherapy has changed the way that cancers are treated. For patients with end-stage HCC, immune checkpoint inhibitor (ICI)-based therapy has recently become the new standard of care [9]. Although evidence suggests that the interactions between tumor cells and immune cells in the tumor microenvironment are responsible for the efficacy of ICI-based therapies, these interactions remain poorly understood [10]. The various components of the HCC microenvironment, such as infiltrating immune cells, non-immune stromal cells, and secreted cytokines, work together to recruit immune cells into the HCC tissue, which forms the tumor immune microenvironment of HCC [10]. The main immunosuppressive cells in HCC are Kupffer cells, neutrophils, regulatory T cells (Tregs), and myeloid-derived suppressor cells (MDSCs). Also, CD8⁺ cytotoxic T lymphocytes (CTLs), natural killer (NK) cells, and dendritic cells (DCs) are the major cell types for immunosurveillance. In this review, the immune microenvironment of HCC, including the role of effector cells and regulatory or immunosuppressive cells, will be briefly summarized, and ICI-based therapies against HCC will be discussed.

2. The immune microenvironment of liver cancer

The various components of the HCC microenvironment (**Figure 1**), such as infiltrating immune cells, non-immune stromal cells, and secreted cytokines, work together to recruit immune cells into the HCC tissue, thus forming the tumor immune

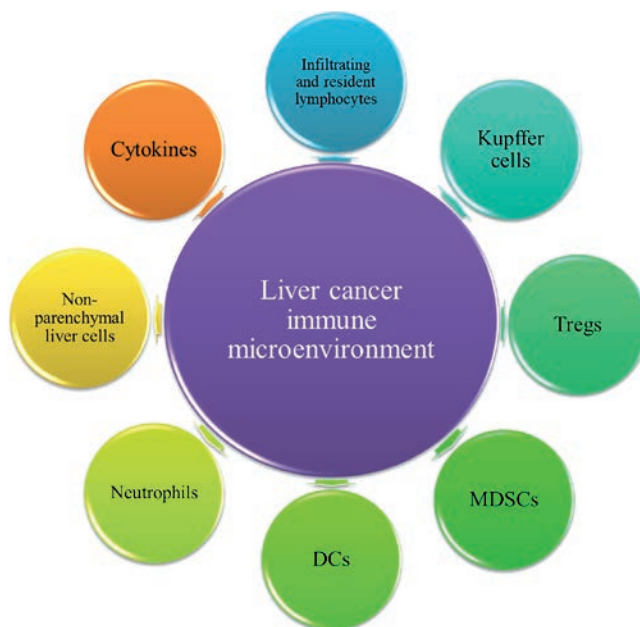


Figure 1.
The schematic diagram of the immune microenvironment in liver cancer.

microenvironment of HCC [10]. Both innate and adaptive immune responses are involved in the antitumor response [11]. An overview of these cells and their role in HCC is provided in **Table 1**.

Natural killer (NK) cells, as the first-line effectors of the innate immune system, recognize targets by a complex array of surface receptors [12]. The downregulation of class I human leukocyte antigen (HLA) ligands on inhibitory receptors on tumor cells triggers the antitumor response of NK cells [12]. In addition, the expression of the stress-induced protein ligands of activating NK receptors, toll-like receptor ligands, and cytokines also affects NK cell activity [12].

Activated DCs present processed tumor-associated antigens (TAAs), which are bound to class II HLA molecules, to naïve CD4⁺ T cells, triggering the adaptive immune response [10]. The binding of antigen and HLA class II complex promotes CD4⁺ T cells to differentiate into interferon-gamma (IFN γ)-producing type 1 T helper (Th1) cells [10]. Th1 cells assist DCs to present antigens on HLA class I to CD8⁺ T cells, inducing their differentiation into effector CD8⁺ cytotoxic T lymphocytes (CTLs) [6]. CTLs release granzyme B, perforin, and IFN γ and mediate Fas/FasL interaction to kill tumor cells [13]. Several lines of evidence indicate that the interplay between

Cell type	Characteristics in HCC	Reference
Dendritic cells	Present antigens to CD8 ⁺ T cells to induce the differentiation of effector CD8 ⁺ cytotoxic T lymphocytes.	[6]
Natural killer cells	The first-line effectors of the innate immune system, the immature NK cell set, are functional impairments and were associated with worse HCC patient survival.	[12] [23, 24]
CD8 ⁺ T cells	Active immune recognition of tumor cells and is associated with better survival. Exhibit an exhausted phenotype and transit into memory T cells with impairment.	[20] [25]
Regulatory T cells	Inhibit IL2-mediated T-cell activation. Produce immunosuppressive molecules, including IL-10 and TGF- β . Impair the effector function of CD8 ⁺ T cells.	[26] [27] [28]
CD4 ⁺ invariant natural killer T cells	Inhibit the expansion of tumor antigen-specific CD8 ⁺ T cells.	[29]
Myeloid-derived suppressor cells	Dampen NK cell and T cell immune function. Differentiate into macrophages and prevent HCC initiation. Recruit Tregs	[30] [31] [31]
Neutrophils	Inhibit the antitumor response of T cells. Recruit CCR2 ⁺ macrophages and CCR4 ⁺ Tregs.	[33] [34]
Kupffer cells	Augment HCC cell proliferation. Inhibit the antitumor response of T cells.	[38] [39]
Hepatic stellate cells	Suppress CD8 ⁺ T-cell function through the recruitment of Tregs and MDSCs.	[45, 47]
Liver sinusoidal endothelial cells	Inhibit active T-cell function.	[46]
Cancer-associated fibroblasts	Impair NK cell function. Promote the MDSC generation.	[48] [49]

Table 1.
The cell types and characteristics of immune cells in the HCC microenvironment.

tumor cells and tumor immune microenvironment components is a key factor for the evolution of liver cancer, especially HCC, and for the response to immunotherapies [14]. The liver harbors a substantial reservoir of immune cells, including resident macrophages (Kupffer cells), NK cells, neutrophils, NK T cells, and monocytes, as well as lymphocytes that either transit through or reside within the liver [15]. These lymphocytes encompass CD4⁺ T cells, CD8⁺ T cells, $\gamma\delta$ T cells, and B cells [15].

Circulating HBV or HBV-derived antigens during chronic HBV infection would promote T-cell exhaustion and subsequent death, which would weaken the global immunity of the patient [16]. HCV, on the other hand, evades immune surveillance because of the high mutation rate and counteracting DNA sensors through viral factors [17]. The majority of HCC patients were infected with HBV or HCV, making HCC evolve in this chronic immunosuppressive environment [15]. Decreases in T-cell costimulatory factors lead to an increase in immune-checkpoint molecules, resulting in impaired T cell effector functions [18]. Additionally, a number of different immune evasion mechanisms were also present in HCC [9].

Canonically, CD8⁺ T cells recognize tumor-related epitopes and other peptide antigens, presented on major histocompatibility complex (MHC) class I molecules [19]. Subsequently, they can produce cytokines and cytolytic molecules after being activated and differentiated into effector T cells, which enable them to eliminate tumor cells directly [19]. The infiltration of cytotoxic CD8⁺ T cells into the liver tumor microenvironment suggests active immune recognition of tumor cells, which is associated with better survival [20]. However, in the context of persistent antigen stimulation and immunoregulatory cues, CD8⁺ T cells often become exhausted, and the effector function is reduced [20].

It was found by van Buuren et al. that two unique liver immune microenvironments classified as immune high and immune low were found through biopsies of the livers of HCC patients with HBV infection [21]. After conducting a differential gene expression analysis, it was observed that these two distinct liver immune microenvironments exhibit varying expressions of immune genes. Specifically, it was demonstrated that the majority of immune genes are upregulated in the immune high microenvironment [21]. In addition, various immune pathways, including interferon and other inflammatory signaling pathways, were upregulated in the high-immune microenvironment. The immune high microenvironment showed increased cell signatures representing T cells, B cells, and monocytes and a decrease in hepatocyte signatures [21]. Furthermore, the transcriptome analysis indicated a significantly increased number of T cells, B cells, and macrophages in the immune-rich microenvironment [21]. Multiplex immunofluorescence also confirmed intrahepatic CD4⁺ T cells, CD8⁺ T cells, B cells, and CD68⁺ macrophages [21]. Significantly increased expression of immune checkpoint genes such as programmed cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), cytotoxic T-lymphocyte protein 4 (CTLA4), T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibition motif domain (TIGIT), and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) was found in the immune high microenvironment [21]. However, elevated expression of checkpoint receptors and ligands during chronic infection is typically associated with T-cell exhaustion and dysfunctional immune responses [21]. T-cell exhaustion represents a common mechanism of tumor immune evasion [22]. Exhausted CD8⁺ T cells accumulate in chronic infection or cancer because of persistent antigen stimulation as well as excessive exposure to inhibitory signals [22]. The impairment of T-cell effector function contributes to immune-mediated tumor therapeutic failure [22]. Exhausted CD8⁺ T cells have a distinct epigenetic landscape compared to functional effector and memory

T cells, as well as an altered transcriptional program that includes high expression of the transcription factor thymocyte selection-associated high mobility group box protein (TOX) [22].

2.1 NK cells

Immature CD11-CD27-NK cells were found to accumulate more in HCC tissue compared to adjacent non-tumor tissue or normal liver [23]. The immature NK subset was inactive, the cytotoxicity was decreased, and IFN γ -producing capacity was impaired [23]. This dysfunctional NK cell subset was associated with worse HCC patient survival [23].

NK cell function impairment has also been attributed to defective interactions between their receptors and ligands. NKG2D receptors are expressed in NK cells, and their ligands, MHC-associated chains A and B (MICA and MICB), are expressed by HCC cells [24]. HCC cells can escape from NK cell immunological surveillance through shedding soluble MICA to down-regulate the NKG2D receptor in NK cells [24]. Additionally, ULBP1, another ligand of NKG2D, was decreased in HCC, which caused immune evasion and is correlated to early HCC recurrence [24].

2.2 Infiltrating T cells

The tumor-infiltrating T-cell number has been closely correlated to the survival of HCC patients [25]. More CD4⁺ and CD8⁺ T cells infiltrate into HCC in the early stage than in the late stage, and their numbers decrease during HCC progression [25]. CD8⁺ T cells exhibit an exhausted phenotype, upregulate co-inhibitory receptors, and subsequently transition into memory T cells with impairment [25].

It was evidenced that the number of regulatory T cells (Tregs) in HCC tissue and peripheral blood was inversely correlated to the overall survival [26]. Tregs are CD4⁺CD25⁺FoxP3⁺ T cells, which play an inhibitory role in T-cell immune function [26]. CD25, the interleukin (IL) 2 receptor α chain, can bind to IL2 with high affinity, which inhibits IL2-mediated T-cell activation [26]. CTLA4 expression is constitutively expressed in Tregs, and the immune-suppressive molecules including IL10 and TGF β are also produced by Tregs [27]. The infiltrating Tregs in HCC have been proven to impair the effector function of CD8⁺ T cells and promote tumor progression [28].

Invariant NK T (iNKT) cells are T lymphocytes with shared properties of both T cells and NK cells [29]. CD4⁺ iNKT cells were reported to be enriched in liver cancer. Their presence limited the expansion of tumor antigen-specific CD8⁺ T cells through the production of Th2 cytokines [29].

2.3 Myeloid-derived suppressor cells (MDSCs)

MDSCs are immature myeloid progenitor cells dampening NK cell and T-cell immune function in the tumor environment [30]. They suppress T-cell proliferation by depleting arginine and releasing reactive oxygen and nitrogen species that disrupt TCR signaling [30].

MDSCs, which are recruited by senescent hepatocytes, can differentiate into macrophages and prevent HCC initiation [31]. On the contrary, after the development of HCC, they lose their differentiation capacity [31]. Additionally, CD14-positive and HLA-DR-negative MDSCs accumulate in the tumor microenvironment of HCC and

can recruit Tregs [31]. The MDSC subset has been reported to be a prognostic factor for HCC progression and recurrence [32].

2.4 Neutrophils

Neutrophils in the tumor microenvironment induce tumor proliferation, promote angiogenesis, and inhibit the antitumor response of T cells [33]. The neutrophils to lymphocyte ratio negatively correlates to the HCC patient survival [34]. Moreover, infiltrating neutrophils can recruit CCR2⁺ macrophages and CCR4⁺ Tregs in HCC [34]. The number of these cells has been identified as a negative prognostic factor in HCC patients undergoing curative resection [35].

2.5 Kupffer cells

Liver macrophages consist of distinct populations termed KCs and monocyte-derived macrophages. KCs express the C-Type Lectin Domain Family 4 Member F (CLEC4F) uniquely and can be characterized as CD11b⁺, F4/80⁺, TIM4⁺, and CLEC4F⁺ cell populations [36]. Liver macrophages are very plastic and adapt their phenotype according to signals of the hepatic microenvironment, which explains their manifold and even opposing functions during HCC [37].

It has been documented that KCs release different mediators, including IL-6, IL-1 β , CCL2, VEGFA, and TNF, to augment tumor cell proliferation in HCC. Also, KCs have been demonstrated to function as incomplete antigen-presenting cells (APCs) to induce T-cell tolerance [38]. They accumulate at the peritumoral stroma and inhibit the antitumor response by expressing high levels of PD-L1 and galectin-9 to impede the activating PD-L1/PD-1 and galectin-9/TIM-3 signaling in the T cells [39]. Moreover, KCs secrete IL-10, indoleamine 2–3 dioxygenase (IDO), and upregulate PD-L1 to take part in the immune inhibition process [40]. The interaction between PD-L1 on Kupffer cells and PD-1 on activated T cells is implicated in T-cell dysfunction in HCC [41].

Nevertheless, macrophages also participate in HCC tumor surveillance. CCR2⁺ macrophages and T-cell activation result in the clearance of senescent hepatocytes, thereby preventing HCC development [42]. However, the recruitment of CCR2⁺ macrophages leads to immune-suppressive tumor-associated macrophages (TAMs) or MDSC accumulation, which inhibits the activation of CD8⁺ T cells and NK cells to favor tumor growth [42].

2.6 Non-parenchymal liver cells

Non-parenchymal liver cells such as hepatic stellate cells (HSCs), liver sinusoidal endothelial cells (LSECs), and cancer-associated fibroblasts (CAFs) are also involved in the antitumor immune response modulation [43]. LSECs as well as HSCs express PD-L1 to inhibit active T-cell function and induce the accumulation of Tregs [44]. The overexpression of PD-L1 on LSECs inhibits the activation of CD8⁺ T cells and leads to immune evasion of HCC [45]. At first, LSECs present MHC I-restricted soluble antigens to CD8⁺ T cells to initially stimulate naïve CD8⁺ T cells; however, subsequent overexpression of co-inhibitory B7-H1 molecules on LSECs induced the tolerance of CD8⁺ T cells toward tumor antigens [46]. HSCs can also be activated by KCs, contributing to extracellular matrix remodeling and stiffness, as well as generating an immunosuppressive microenvironment to

suppress CD8⁺ T cell function through the recruitment of Tregs and MDSCs [47]. CAFs in HCC secrete prostaglandin E2 and IDO to impair NK cell function [48]. In addition, the secretion of IL-6 and stromal cell-derived factor 1 α -CXCL12 in CAFs promotes the MDSC generation [49].

2.7 Cytokines

An array of cytokines present in the HCC microenvironment plays a significant role in tumor evolution [9]. Both parenchymal and non-parenchymal cells, as well as infiltrating immune cells, are capable of secreting cytokines [9].

Several cytokines in the HCC tumor microenvironment are involved in HCC progression [9]. Among them, IL-6 promotes the proliferation of hepatocytes [50]. A higher level of serum IL-6 is correlated with an increased risk of HCC development and metastasis [50].

IL10 is an anti-inflammatory molecule produced by innate, adaptive immune cells and tumor cells [51]. As for the liver, IL-10 is produced by DCs, Kupffer cells, hepatic stellate cells, liver sinusoidal endothelial cells, MDSCs, and T cells [51]. Increased plasma IL-10 levels in HCC patients have been shown to be associated with poor prognosis [52].

TGF β is produced by parenchymal and non-parenchymal liver cells in the tumor microenvironment and regulates tumor cell proliferation, adhesion, and migration [53]. In the liver tumor microenvironment, TGF β might promote the differentiation of macrophages into pro-tumorigenic M2-type, induce the change of naïve CD4⁺ T cells into Tregs, and inhibit CD8⁺ T as well as NK cell immune function [53]. TGF β also contributes to the development and progression of solid tumors [54]. TGF β could activate hepatic stellate cells and is involved in the progression of liver inflammation and fibrosis [54]. Hepatic stellate cells in turn are the main producers of TGF β [30]. In the early stages of hepatocarcinogenesis, TGF β inhibits tumor cell proliferation and promotes apoptosis [55]. However, after tumor progression, TGF β promotes apoptosis resistance and cell proliferation to support tumor progression and dissemination [56]. Thus, the elevated level of plasma TGF β is correlated with decreased patient survival in individuals with unresectable HCC [56].

IDO contributes to the conversion of tryptophan to kynurenine, which is constitutively produced by various cells in the tumor microenvironment, including tumor cells and IFN γ -producing CD8⁺ T cells [57]. In HCC, IDO overexpression is associated with poor prognosis and tumor metastasis [58].

Macrophages can be induced into the HCC tumor microenvironment and differentiated into a pro-tumorigenic phenotype by colony-stimulating factor 1 [59]. It was reported that PLX3397 could block the colony-stimulating factor 1 receptor, inducing the shift of macrophages toward an M1-like phenotype and promoting CD8⁺ T-cell infiltration [60]. This inhibition ultimately suppressed HCC tumor growth [60].

3. Immunotherapy of HCC

The immune-based approaches against HCC have been tested for many years, mostly focusing on vaccination strategies, cytokines, or non-specific T-cell activation, with most yielding disappointing results [61]. Nevertheless, with the FDA approval of ICIs for cancer treatment, ICI-based therapy sheds light on HCC patients [62].

3.1 ICI-based therapy

Immune cells, including B cells, T cells, DCs, monocytes, NK cells, TAMs, and MDSCs, express immune checkpoints, which are specific subtypes of membrane-bound molecules modulating the immune response [63].

Immune checkpoints display an immunosuppressive activity, which prevents uncontrolled and continuous T-cell effector function upon initial stimulation to limit collateral tissue damage [64]. Among them, PD-1, CTLA-4 (cytotoxic T-lymphocyte protein 4), TIM-3, lymphocyte activation gene 3 (LAG-3) protein, and B- and T-lymphocyte attenuator (BTLA) are the most studied immune checkpoint molecules in cancer research [64].

PD-1 is encoded by the *Pdcd1* gene, which is located on chromosome 2 (2q37) [65]. PD-1 is the type I transmembrane protein of the immunoglobulin superfamily, which contains an extracellular N-terminal IgV-like domain, a transmembrane domain, and a cytoplasmic tail [66]. PD-1 is expressed by activated CD4⁺ and CD8⁺ T cells, B cells, DC, NK cells, Treg, MDSC, and monocytes and plays a key role in the effector phase of the immune response [67]. The ligands of PD-1 are PD-L1 and PD-L2 [67].

The PD-L1 gene is located on chromosome 9p24.1. PD-L1 is a heavily glycosylated protein, consisting of IgV and IgC domains. PD-L1 is primarily expressed in hematopoietic cells, including antigen-presenting cells (APCs) and MDSCs, and is also found in various types of parenchymal cells [67]. The expression of PD-L2 is limited to hematopoietic cells [67]. PD-1 can block TCR signaling to inhibit CD8⁺ T-cell activation upon binding to PD-L1 or PD-L2 [68]. In addition, the binding of PD-1 and its ligands can inhibit CD4⁺ T-cell activation and proliferation by secreting elevated IL-10 [68]. Cancer cells can evade immune surveillance by expressing PD-L1 and PD-L2 to inhibit the function of CD8⁺ and CD4⁺ T cells [68]. In the tumor microenvironment, sustained chronic exposure to TAAs prompts T cells specific to these antigens to produce IFN- γ , thereby inducing PD-1 expression on reactive T lymphocytes [69]. However, the expression of PD-L1 increases in APCs and tumor cells. The enhanced PD-1-PD-L1 engagements block T-cell receptor (TCR) signaling, inhibiting T-cell proliferation and cytotoxic mediator secretion, ultimately leading to T-cell exhaustion [69].

At the priming phase of CD4⁺ T-cell activation, CTLA-4 is essential [70]. The CTLA-4 gene is localized on chromosome 2q33, which consists of four exons [71]. The peptide leader, ligand-binding domain or IgV-like domain, transmembrane domain, and cytoplasmic tail of the CTLA-4 protein are encoded by exon 1, exon 2, exon 3, and exon 4, respectively [72]. CTLA-4 is expressed on activated T cells and exhibits high affinity for CD80 and CD86, leading to reduced interaction with CD28, thereby inhibiting T cell activation during antigen presentation. Tregs are CD4⁺ T cells, characterized by the presence of CD25, FoxP3, CTLA-4, and CD62L [70]. Tregs constitutively express CTLA-4 to exert suppressive activity against activated T cells in the tumor microenvironment [70]. Tregs inhibit the immune response through various mechanisms, including (1) depletion of IL-2 and secretion of immunosuppressive factors such as TGF- β , IL-10, and adenosine and (2) competition with costimulatory CD28 via CTLA-4 [70].

The TIM gene is located on human chromosome 5q33.2 [73]. TIM-3 belongs to the TIM family of type-I cell-surface glycoproteins, which contains a conserved extracellular IgV domain with N-linked glycosylation sites, a transmembrane domain, and an intracellular cytoplasmic tail with 5 tyrosines [74]. TIM-3 is broadly expressed in innate and adaptive immune cells and interacts with various ligands, including

galectin-9, phosphatidylserine, and others [75]. Galectin-9 is a soluble protein and was confirmed to suppress T-cell responses [75]. Hence, TIM-3 is verified to act as an inhibitory receptor for T cells from another perspective [75]. Furthermore, CD8 and TIM-3 double-positive T cells co-express PD-1 in animal models [75]. These cells exhibit greater defects in terms of cell-cycle progression and production of effector cytokines compared to those expressing PD-1 alone [75]. Thus, TIM-3 may cooperate with PD-1 to produce severe dysfunctional CD8⁺ T-cell phenotype in cancer [76].

Lymphocyte activation gene-3 (LAG3) is a potential cancer immunotherapeutic target due to its negative regulatory role on T cells and its capacity, in combination with PD-1, to mediate a state of exhaustion [77]. LAG3 is a 55 kDa type I transmembrane glycoprotein comprising four extracellular immunoglobulin (Ig)-like domains (D1–D4) [78], a connecting peptide, and an intracellular region that transmits inhibitory signals to the T cells upon binding to MHC class II and other ligands [79]. LAG-3 is a marker of exhausted T cells and is not expressed on resting T cells, and its expression increases upon the activation of T cells [80]. LAG-3 plays a synergistic role with PD-1 in aiding cancer cells to evade the immune system [80].

The BTLA gene is located on chromosome 3 in q13.2 in reverse orientation and encodes a 289 amino acid long type 1 transmembrane glycoprotein (33 kD) [81]. The BTLA protein consists of a signal peptide, an IgC-like extracellular domain, a transmembrane domain, and a cytoplasmic domain [82]. Several immune cells, including T and B lymphocytes, DCs, and NK cells, express the immunoglobulin-like molecule BTLA [83]. BTLA also inhibits T-cell proliferation and cytokine production upon binding to its ligand [84].

ICIs disrupt the interaction between tumor cells and T cells to restore the antitumor response of T cells and have contributed to a partial improvement in the survival rates of HCC patients [85]. Currently approved ICIs for HCC treatment by the FDA are nivolumab, pembrolizumab, atezolizumab, durvalumab, tremelimumab, tislelizumab, ipilimumab, and sintilimab [86]. Camrelizumab was approved by the Chinese Food and Drug Administration in 2020 and is expected to be approved by the FDA in the near future [86].

Nivolumab, an anti-PD-1 IgG4 mono antibody, was approved by the FDA in 2017 as a second-line therapy for HCC patients after the failure of sorafenib treatment [87]. In the phase I/II clinical trial CheckMate 040, which enrolled HCC patients with varying Child-Pugh scores, nivolumab demonstrated a manageable toxicity profile and significantly reduced tumor burden [88]. In the randomized, multicenter, open-label, phase III trial CheckMate 459, the efficacy of nivolumab was compared with that of sorafenib [89]. It was shown that drug-related adverse events were reported in 12% of patients receiving nivolumab and 11% of patients receiving sorafenib [89]. The objective response rate was 15% in the nivolumab group and 7% in the sorafenib group, respectively. However, the overall survival in the nivolumab group did not reach the pre-defined statistical significance threshold, suggesting that nivolumab did not improve overall survival over sorafenib [89].

Pembrolizumab, which was approved by the FDA in 2018 as a PD-1 inhibitor, was evaluated in KEYNOTE-224 and KEYNOTE-240 in the second-line setting [90, 91]. In a phase II KEYNOTE-224 trial, the overall survival and progression-free survival of patients treated with pembrolizumab did not reach statistical significance according to the pre-specified criteria [90]. KEYNOTE-240 was a phase III trial conducted in advanced HCC patients previously treated with sorafenib, who were randomized to receive either pembrolizumab or a placebo [92]. The median overall survival and progression-free survival of patients in the pembrolizumab group were 13.9 months

and 3 months, respectively, compared to 10.6 months and 2.8 months in the placebo group [92]. The toxicity profile of pembrolizumab was deemed acceptable, with 18.6% of patients experiencing treatment-related adverse events, including seven deaths [92].

Camrelizumab, another anti-PD-1 antibody, was evaluated in a multicenter randomized phase II trial for the treatment of Chinese HCC patients [93]. Camrelizumab, as a second-line agent for patients with advanced HCC, showed an acceptable toxic profile with an objective response rate of 14.7% [93].

Atezolizumab is an engineered IgG1 monoclonal antibody designed to inhibit PD-L1 specifically [94]. It was observed that the single-agent atezolizumab yielded a median progression-free survival of 3.4 months with only mild adverse effects [94]. A randomized phase Ib trial showed that the combination of atezolizumab and bevacizumab led to improved progression-free survival in HCC patients, with a manageable adverse reaction profile, compared to atezolizumab monotherapy [95].

Durvalumab, another anti-PD-L1 monoclonal antibody, has been administered to unresectable HCC patients previously treated with sorafenib [59]. Durvalumab was non-inferior to sorafenib for overall survival in a pre-specified non-inferiority secondary endpoint analysis [96].

Tremelimumab is an anti-CTLA4 antibody [97]. The results of a phase I/II clinical trial showed that tremelimumab achieved an overall response rate of 7.2%, with a median overall survival of 15.1 months for patients diagnosed with unresectable HCC. Furthermore, patient response was found to be closely associated with the early expansion of CD8⁺ T cells [98].

3.2 ICI-based combination therapy

Although ICI monotherapy has shown promising results in treating HCC, response rates remain low [99]. In the hepatocellular carcinoma (HCC) tumor micro-environment, multiple immunosuppressive factors exist. Therefore, it is unlikely that ICI monotherapy, which targets only one active site, will substantially improve the outcome for HCC patients [100]. Combining immune checkpoint inhibitors (ICIs) with tyrosine kinase inhibitors (TKIs) or anti-VEGF monoclonal antibodies, as well as employing dual ICI therapy, is likely to yield better patient outcomes compared to single immune checkpoint blockade [101]. The IMbrave150 trial evaluated the combination of atezolizumab and the VEGF inhibitor bevacizumab in comparison to TKI monotherapy, with the aim of improving overall survival in advanced HCC patients. This combination was approved by the FDA in 2020 [101]. Atezolizumab in combination with bevacizumab has become standard first-line systemic therapy for patients with untreated advanced HCC, which is significantly superior to sorafenib [97].

The combination of ICIs nivolumab and ipilimumab, which inhibit the PD-1 and CTLA-4 pathways respectively, was approved by the FDA in 2020 as a second-line treatment for patients with advanced HCC [102]. The CheckMate 040 trial utilized various dosages of nivolumab and ipilimumab to treat advanced HCC patients who had previously received sorafenib. The trial showed promising results with manageable safety and an objective response rate of 32% [102].

Other therapies that combine ICIs with locoregional therapy, TKIs, or chemotherapy are also under clinical investigation [64]. After administering tremelimumab following local ablative therapy, 26.3% of the HCC patients achieved a confirmed partial response, accompanied by an increase in the number of intra-tumor CD8⁺ cells [103]. The probabilities of tumor progression-free survival at 6 and 12 months were 57.1% and 33.1%, respectively [103].

The combination of pembrolizumab with lenvatinib showed promising antitumor activity with manageable toxicities and no unexpected safety signals in a phase Ib study involving 104 unresectable HCC patients [104].

Camrelizumab combined with a standard FOLFOX4 (infusional fluorouracil, leucovorin, and oxaliplatin) regimen conferred an objective response and disease control rate of 29.4% and 79.4%, respectively, in 34 patients with advanced HCC in a multicenter phase Ib/II clinical trial [105]. The combined therapeutic approach employed as the initial treatment regimen exhibited encouraging preliminary antitumor efficacy alongside favorable safety profiles and tolerability [105].

3.3 Innovative immunotherapy strategies

With the advancement of CRISPR-Cas9 technology, the generation of chimeric antigen receptor (CAR)-T cells has been revolutionized [106]. CAR-T therapy requires the genetic engineering of the cancer patient's own T cells [107]. When specific antigens are recognized, CAR-T cells undergo significant expansion and can infiltrate the tumor microenvironment to target tumor cells with high affinity [107]. However, the inhibitory receptors on T cells, including PD-1, CTLA-4, and LAG-3, can impede CAR-T efficiency [108]. Although no CAR T-cell therapy for HCC has been approved by the FDA, some CARs against HCC have been developed [109]. CAR-NK cells were also used to improve the targetability and efficacy of NK cells. In HCC, CAR-modified NK-92 cells were used to specifically target Glypican-3 (GPC3)-positive tumor cells [110].

Several T cell receptor (TCR) engineered T cells (TCR-T) therapies were approved for clinical use in the treatment of liver cancer. In a phase I clinical trial, HBV DNA levels were reduced in most patients after treatment with TCR-T cells with advanced hepatitis B HCC [111]. The mRNA-HBV-TCR-T cells were well tolerated in the treatment of HCC patients with HBV infection and showed long-term clinical benefits [112].

Naturally occurring and genetically modified oncolytic viruses are designed to replicate in tumor cells while sparing normal cells, thus targeting tumor cells specifically [113]. The use of the JX-594 vaccinia virus as an oncolytic virus, exhibiting oncolytic activity, was found to be safe and demonstrated a dose-related survival benefit in advanced HCC patients [114]. However, in a subsequent phase IIb trial, Pexa-Vec did not improve the overall survival of advanced HCC patients treated with sorafenib previously [115].

Cancer vaccines can activate the adaptive immune system of the patient to eliminate tumors [116]. A study demonstrated that a cancer vaccine containing personalized neoantigen peptides improved recurrence-free survival among postoperative HCC patients [117].

A Phase I trial involving 17 patients suggested that intra-tumor injection of the immune-priming agent, ilixadencel, a pro-inflammatory allogeneic DC, into patients with hepatocellular carcinoma (HCC) elicited tumor-specific immunologic responses [118]. In a phase I/II trial, the intradermal administration of HepaVac-101-a combined peptide antigen and an RNA-based immunostimulator into early to immediate-stage HCC patients was found to be safe and effective [117].

Adoptive cell therapy (ACT) is also known as cellular immunotherapy. Adoptive transfer of NK cells from peripheral blood in combination with HCC cryoablation improved global immune function and prolonged the progression-free survival of advanced HCC patients [119]. NK cells can be derived from stem cells, from peripheral blood NK cells of a healthy donor (allogeneic condition), from the patient (autologous condition), or from the NK-92 cell line.

A phase I study has reported that ACT of allogeneic cytokine-induced killer (CIK) cells prevented HCC recurrence after liver transplantation [120]. Moreover, two phase III clinical trials have demonstrated that NK-based CIK therapies were well tolerated and improved the overall survival of advanced HCC patients [121, 122].

4. Conclusions and future perspectives

Immunotherapy has opened a new era and remains an active area of research in treating advanced HCC. Many clinical trials are ongoing to evaluate ICIs and ICI-based combination therapies. Despite these unprecedented and encouraging results, only 20–30% of patients respond to immunotherapies, and so far biomarkers have failed to clearly elucidate the responding groups [123]. The presence of multiple alternative pathways and factors can bypass ICIs. Moreover, some patients develop immune resistance after successful ICI therapy. The potential causes of immune resistance in HCC patients to ICI-based therapies are complex and diverse. Resistance to ICI-based therapy might be attributed to impairment in antigen recognition and presentation, abnormal activation and proliferation of immunosuppressive cells, increased secretion of inhibitory cytokines and chemokines, and exhaustion of cytotoxic T cells. Additionally, tumor antigen deletion, tumor heterogeneity, and gut microbiota collectively contribute to resistance against ICI-based therapies. Nevertheless, the mechanisms of ICI-based therapy resistance are not fully elucidated. Better insight into the complex immune microenvironment of HCC may further help us pave the way to new therapeutic options. Despite these challenges, ongoing research offers a promising path toward more efficacious and safer innovative therapies. Undoubtedly, the convergence of immunotherapeutic modalities, coupled with the continuous progress in scientific investigation, holds promise to advance HCC patients' care.

The current mainstream combination therapies, which involve combining ICIs with other ICIs, TKIs, targeted therapy, chemotherapy, and local-regional therapy, have demonstrated better outcomes. Therefore, the incorporation of immunotherapy combinations into the HCC landscape is changing the natural history of the HCC and will be the backbone of future drug development.

Acknowledgements

This work was supported by the National Natural Science Foundation of China, grant no. 82303572, the Science and Technology Department of Liaoning Province, grant no. 2024-MS-285, and the Dalian Science and Technology Bureau, grant no. 2023ZXYG33.

Conflict of interest

The authors declare no conflict of interest.

Author details

Chang Liu*, Yi-Jun Tu and Li Zhang
Central Hospital of Dalian University of Technology, Dalian, China

*Address all correspondence to: liuch2024@dlut.edu.cn

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*. 2024;**74**(3):229-263. DOI: 10.3322/caac.21834
- [2] Plummer M, de Martel C, Vignat J, et al. Global burden of cancers attributable to infections in 2012: A synthetic analysis. *The Lancet Global Health*. 2016;**4**:e609-e616. DOI: 10.1016/S2214-109X(16)30143-7
- [3] Gilles H, Garbutt T, Landrum J. Hepatocellular Carcinoma. *Critical Care Nursing Clinics of North America*. 2022;**34**:289-301. DOI: 10.1016/j.cnc.2022.04.004
- [4] Ettrich TJ, Seufferlein T. Regorafenib. *Recent Results in Cancer Research*. 2018;**211**:45-56. DOI: 10.1007/978-3-319-91442-8_3
- [5] Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *The New England Journal of Medicine*. 2008;**359**:378-390. DOI: 10.1056/NEJMoa0708857
- [6] Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. *Nature Reviews. Disease Primers*. 2021;**7**(1):6. DOI: 10.1038/s41572-020-00240-3
- [7] Yamashita T, Kudo M, Ikeda K, et al. REFLECT-a phase 3 trial comparing efficacy and safety of lenvatinib to sorafenib for the treatment of unresectable hepatocellular carcinoma: An analysis of Japanese subset. *Journal of Gastroenterology*. 2020;**55**:113-122. DOI: 10.1007/s00535-019-01642-1
- [8] Bruix J, Qin S, Merle P, et al. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): A randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2017;**389**:56-66. DOI: 10.1016/S0140-6736(16)32453-9
- [9] Donne R, Lujambio A. The liver cancer immune microenvironment: Therapeutic implications for hepatocellular carcinoma. *Hepatology*. 2023;**77**:1773-1796. DOI: 10.1002/hep.32740
- [10] Cariani E, Missale G. Immune landscape of hepatocellular carcinoma microenvironment: Implications for prognosis and therapeutic applications. *Liver International*. 2019;**39**:1608-1621. DOI: 10.1111/liv.14192
- [11] Ruf B, Heinrich B, Greten TF. Immunobiology and immunotherapy of HCC: Spotlight on innate and innate-like immune cells. *Cellular & Molecular Immunology*. 2021;**18**:112-127. DOI: 10.1038/s41423-020-00572-w
- [12] Jinushi M, Takehara T, Tatsumi T, et al. Impairment of natural killer cell and dendritic cell functions by the soluble form of MHC class I-related chain A in advanced human hepatocellular carcinomas. *Journal of Hepatology*. 2005;**43**:1013-1020. DOI: 10.1016/j.jhep.2005.05.026
- [13] Yuen MF, Hughes RD, Heneghan MA, et al. Expression of Fas antigen (CD95) in peripheral blood lymphocytes and in liver-infiltrating, cytotoxic lymphocytes in patients with hepatocellular carcinoma. *Cancer*. 2001;**92**:2136-2141. DOI: 10.1002/1097-0142(20011015)92:8<2136::aid-cncr1555>3.0.co; 2-j

- [14] Liu Y, Xun Z, Ma K, et al. Identification of a tumour immune barrier in the HCC microenvironment that determines the efficacy of immunotherapy. *Journal of Hepatology*. 2023;78:770-782. DOI: 10.1016/j.jhep.2023.01.011
- [15] Ringelhan M, Pfister D, O'Connor T, et al. The immunology of hepatocellular carcinoma. *Nature Immunology*. 2018;19:222-232. DOI: 10.1038/s41590-018-0044-z
- [16] Ye B, Liu X, Li X, et al. T-cell exhaustion in chronic hepatitis B infection: Current knowledge and clinical significance. *Cell Death & Disease*. 2015;6:e1694. DOI: 10.1038/cddis.2015.42
- [17] Li XD, Sun L, Seth RB, et al. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102:17717-17722. DOI: 10.1073/pnas.0508531102
- [18] Kassel R, Cruise MW, Iezzoni JC, et al. Chronically inflamed livers up-regulate expression of inhibitory B7 family members. *Hepatology*. 2009;50:1625-1637. DOI: 10.1002/hep.23173
- [19] Hofmann M, Tauber C, Hensel N, et al. CD8+ T cell responses during HCV infection and HCC. *Journal of Clinical Medicine*. 2021;10:991. DOI: 10.3390/jcm10050991
- [20] Itoh S, Yoshizumi T, Yugawa K, et al. Impact of immune response on outcomes in hepatocellular carcinoma: Association with vascular formation. *Hepatology*. 2020;72:1987-1999. DOI: 10.1002/hep.31206
- [21] van Buuren N, Ramirez R, Turner S, et al. Characterization of the liver immune microenvironment in liver biopsies from patients with chronic HBV infection. *JHEP Reports*. 2021;4:100388. DOI: 10.1016/j.jhepr
- [22] Wang X, He Q, Shen H, et al. TOX promotes the exhaustion of antitumor CD8+ T cells by preventing PD1 degradation in hepatocellular carcinoma. *Journal of Hepatology*. 2019;71:731-741. DOI: 10.1016/j.jhep.2019.05.015
- [23] Zhang QF, Yin WW, Xia Y, et al. Liver-infiltrating CD11b (-) CD27 (-) NK subsets account for NK-cell dysfunction in patients with hepatocellular carcinoma and are associated with tumor progression. *Cellular & Molecular Immunology*. 2017;14:819-829. DOI: 10.1038/cmi.2016.28
- [24] Kamimura H, Yamagiwa S, Tsuchiya A, et al. Reduced NKG2D ligand expression in hepatocellular carcinoma correlates with early recurrence. *Journal of Hepatology*. 2012;56:381-388. DOI: 10.1016/j.jhep.2011.06.017
- [25] Fu J, Zhang Z, Zhou L, et al. Impairment of CD4+ cytotoxic T cells predicts poor survival and high recurrence rates in patients with hepatocellular carcinoma. *Hepatology*. 2013;58:139-149. DOI: 10.1002/hep.26054
- [26] Ormandy LA, Hillemann T, Wedemeyer H, et al. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Research*. 2005;65:2457-2464. DOI: 10.1158/0008-5472.CAN-04-3232
- [27] Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: Mechanisms of differentiation and function. *Annual Review of Immunology*. 2012;30:531-564. DOI: 10.1146/annurev.immunol.25.022106.141623

- [28] Kalathil S, Lugade AA, Miller A, et al. Higher frequencies of GARP (+) CTLA-4 (+) Foxp3(+)T regulatory cells and myeloid-derived suppressor cells in hepatocellular carcinoma patients are associated with impaired T-cell functionality. *Cancer Research*. 2013;**73**:2435-2444. DOI: 10.1158/0008-5472.CAN-12-3381
- [29] Bricard G, Cesson V, Devedre E, et al. Enrichment of human CD4+ V(alpha)24/ Vbeta11 invariant NKT cells in intrahepatic malignant tumors. *Journal of Immunology*. 2009;**182**:5140-5151. DOI: 10.4049/jimmunol.0711086
- [30] Kumar V, Patel S, Tcyganov E, et al. The nature of myeloid-derived suppressor cells in the tumor microenvironment. *Trends in Immunology*. 2016;**37**:208-220. DOI: 10.1016/j.it.2016.01.004
- [31] Gao XH, Tian L, Wu J, et al. Circulating CD14+ HLA-DR-/low myeloid-derived suppressor cells predicted early recurrence of hepatocellular carcinoma after surgery. *Hepatology Research*. 2017;**47**:1061-1071. DOI: 10.1111/hepr.12831
- [32] Mizukoshi E, Yamashita T, Arai K, et al. Myeloid-derived suppressor cells correlate with patient outcomes in hepatic arterial infusion chemotherapy for hepatocellular carcinoma. *Cancer Immunology, Immunotherapy*. 2016;**65**:715-725. DOI: DOI
- [33] Mollinedo F. Neutrophil degranulation, plasticity, and cancer metastasis. *Trends in Immunology*. 2019;**40**:228-242. DOI: 10.1007/s00262-016-1837-2
- [34] Xiao WK, Chen D, Li SQ, et al. Prognostic significance of neutrophil-lymphocyte ratio in hepatocellular carcinoma: A meta-analysis. *BMC Cancer*. 2014;**14**:117. DOI: 10.1186/1471-2407-14-117
- [35] Zhou SL, Zhou ZJ, Hu ZQ, et al. Tumor-associated neutrophils recruit macrophages and T-regulatory cells to promote progression of hepatocellular carcinoma and resistance to Sorafenib. *Gastroenterology*. 2016;**150**:1646-1658. DOI: 10.1053/j.gastro.2016.02.040
- [36] Scott CL, Zheng F, De Baetselier P, et al. Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. *Nature Communications*. 2016;**7**:10321. DOI: 10.1038/ncomms10321
- [37] Tacke F. Targeting hepatic macrophages to treat liver diseases. *Journal of Hepatology*. 2017;**66**(6):1300-1312. DOI: 10.1016/j.jhep.2017.02.026
- [38] You Q, Cheng L, Kedl RM, et al. Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. *Hepatology*. 2008;**48**:978-990. DOI: 10.1002/hep.22395
- [39] Li H, Wu K, Tao K, et al. Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma. *Hepatology*. 2012;**56**:1342-1351. DOI: 10.1002/hep.25777
- [40] Heymann F, Peusquens J, Ludwig-Portugall I, et al. Liver inflammation abrogates immunological tolerance induced by Kupffer cells. *Hepatology*. 2015;**62**:279-291. DOI: 10.1002/hep.27793
- [41] Wu K, Kryczek I, Chen L, et al. Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7-H1/programmed death-1 interactions. *Cancer Research*. 2009;**69**:8067-8075. DOI: 0.1158/0008-5472.CAN-09-0901

- [42] Kang TW, Yevsa T, Woller N, et al. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature*. 2011;**479**(7374):547-551
- [43] Yu MC, Chen CH, Liang X, et al. Inhibition of T-cell responses by hepatic stellate cells via B7-H1-mediated T-cell apoptosis in mice. *Hepatology*. 2004;**40**:1312-1321. DOI: 10.1002/hep.20488
- [44] Dunham RM, Thapa M, Velazquez VM, et al. Hepatic stellate cells preferentially induce Foxp3+ regulatory T cells by production of retinoic acid. *Journal of Immunology*. 2013;**190**:2009-2016. DOI: 10.4049/jimmunol.1201937
- [45] Ihling C, Naughton B, Zhang Y, et al. Observational study of PD-L1, TGF- β , and immune cell infiltrates in hepatocellular carcinoma. *Frontiers in Medicine (Lausanne)*. 2019;**6**:15. DOI: 10.3389/fmed.2019.00015
- [46] Höchst B, Schildberg FA, Böttcher J, et al. Liver sinusoidal endothelial cells contribute to CD8 T cell tolerance toward circulating carcinoembryonic antigen in mice. *Hepatology*. 2012;**56**(5):1924-1933. DOI: 10.1002/hep.25844
- [47] Hsieh CC, Hung CH, Chiang M, et al. Hepatic stellate cells enhance liver cancer progression by inducing myeloid-derived suppressor cells through Interleukin-6 signaling. *International Journal of Molecular Sciences*. 2019;**20**(20):5079. DOI: 10.3390/ijms20205079
- [48] Li T, Yang Y, Hua X, et al. Hepatocellular carcinoma-associated fibroblasts trigger NK cell dysfunction via PGE2 and IDO. *Cancer Letters*. 2012;**318**:154-161. DOI: 10.1016/j.canlet.2011.12.020
- [49] Zhao Q, Huang L, Qin G, et al. Cancer-associated fibroblasts induce monocytic myeloid-derived suppressor cell generation via IL-6/exosomal miR-21-activated STAT3 signaling to promote cisplatin resistance in esophageal squamous cell carcinoma. *Cancer Letters*. 2021;**518**:35-48. DOI: 10.1016/j.canlet.2021.06.009
- [50] Wong VW, Yu J, Cheng AS, et al. High serum interleukin-6 level predicts future hepatocellular carcinoma development in patients with chronic hepatitis B. *International Journal of Cancer*. 2009;**124**:2766-2770. DOI: 10.1002/ijc.24281
- [51] Moore KW, de Waal MR, Coffman RL, et al. Interleukin-10 and the interleukin-10 receptor. *Annual Review of Immunology*. 2001;**19**:683-765. DOI: 10.1146/annurev.immunol.19.1.683
- [52] Chau GY, Wu CW, Lui WY, et al. Serum interleukin-10 but not interleukin-6 is related to clinical outcome in patients with resectable hepatocellular carcinoma. *Annals of Surgery*. 2000;**231**:552-558. DOI: 10.1097/00000658-200004000-00015
- [53] Fujii S, Shimizu K, Shimizu T, et al. Interleukin-10 promotes the maintenance of antitumor CD8(+) T-cell effector function in situ. *Blood*. 2001;**98**:2143-2151. DOI: 10.1182/blood.v98.7.2143
- [54] David CJ, Massagué J. Contextual determinants of TGF- β action in development, immunity and cancer. *Nature Reviews. Molecular Cell Biology*. 2018;**19**:419-435. DOI: 10.1038/s41580-018-0007-0
- [55] Fabregat I, Fernando J, Mainez J, et al. TGF-beta signaling in cancer treatment. *Current Pharmaceutical Design*. 2014;**20**:2934-2947. DOI: 10.2174/13816128113199990591

- [56] Fransvea E, Mazzocca A, Antonaci S, et al. Targeting transforming growth factor (TGF)-betaRI inhibits activation of beta1 integrin and blocks vascular invasion in hepatocellular carcinoma. *Hepatology*. 2009;**49**:839-850. DOI: 10.1002/hep.22731
- [57] Asghar K, Farooq A, Zulfiqar B, et al. Indoleamine 2,3-dioxygenase: As a potential prognostic marker and immunotherapeutic target for hepatocellular carcinoma. *World Journal of Gastroenterology*. 2017;**23**:2286-2293. DOI: 10.3748/wjg.v23.i13.2286
- [58] Munn DH, Mellor AL. IDO in the tumor microenvironment: Inflammation, counter-regulation, and tolerance. *Trends in Immunology*. 2016;**37**:193-207. DOI: 10.1016/j.it.2016.01.002
- [59] Zhu XD, Zhang JB, Zhuang PY, et al. High expression of macrophage colony-stimulating factor in peritumoral liver tissue is associated with poor survival after curative resection of hepatocellular carcinoma. *Journal of Clinical Oncology*. 2008;**26**:2707-2716. DOI: 10.1200/JCO.2007.15.6521
- [60] Ao JY, Zhu XD, Chai ZT, et al. Receptor blockade inhibits tumor growth by altering the polarization of tumor-associated macrophages in hepatocellular carcinoma. *Molecular Cancer Therapeutics*. 2017;**16**:1544-1554. DOI: 10.1158/1535-7163.MCT-16-0866
- [61] Greten TF, Manns MP, Korangy F. Immunotherapy of HCC. *Reviews on Recent Clinical Trials*. 2008;**3**:31-39. DOI: 10.2174/157488708783330549
- [62] Bicer F, Kure C, Ozluk AA, et al. Advances in immunotherapy for hepatocellular carcinoma (HCC). *Current Oncology*. 2023;**30**:9789-9812. DOI: 10.3390/curroncol30110711
- [63] Naimi A, Mohammed RN, Raji A, et al. Tumor immunotherapies by immune checkpoint inhibitors (ICIs); the pros and cons. *Cell Communication and Signaling: CCS*. 2022;**20**:44. DOI: 10.1186/s12964-022-00854-y
- [64] Xu F, Jin T, Zhu Y, et al. Immune checkpoint therapy in liver cancer. *Journal of Experimental & Clinical Cancer Research*. 2018;**37**:110. DOI: 10.1186/s13046-018-0777-4
- [65] Shinohara T, Taniwaki M, Ishida Y, et al. Structure and chromosomal localization of the human PD-1 gene (PDCD1). *Genomics*. 1994;**23**:704-706. DOI: 10.1006/geno.1994.1562
- [66] Zhang X, Schwartz JC, Guo X, et al. Structural and functional analysis of the costimulatory receptor programmed death-1. *Immunity*. 2004;**20**:337-347. DOI: 10.1016/s1074-7613(04)00051-2
- [67] Li Q, Han J, Yang Y, et al. PD-1/PD-L1 checkpoint inhibitors in advanced hepatocellular carcinoma immunotherapy. *Frontiers in Immunology*. 2022;**13**:1070961. DOI: 10.3389/fimmu.2022.1070961
- [68] Liu J, Chen Z, Li Y, et al. PD-1/PD-L1 checkpoint inhibitors in tumor immunotherapy. *Frontiers in Pharmacology*. 2021;**12**:731798. DOI: 10.3389/fphar.2021.731798
- [69] Pauken KE, Wherry EJ. Overcoming T cell exhaustion in infection and cancer. *Trends in Immunology*. 2015;**36**:265-276. DOI: 10.1016/j.it.2015.02.008
- [70] Greten TF, Sangro B. Targets for immunotherapy of liver cancer. *Journal of Hepatology*. 2017;**S0168-8278**:32287-0. DOI: 10.1016/j.jhep.2017.09.007
- [71] Dariavach P, Mattéi MG, Golstein P, et al. Human Ig superfamily CTLA-4

gene: Chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. *European Journal of Immunology*. 1988;**18**(12):1901-1905. DOI: 10.1002/eji.1830181206

[72] Jiang T, Ge LQ, Chen ZT, et al. Effect of cytotoxic T lymphocyte-associated molecule 4 1661 gene polymorphism on its expression and transcription in ulcerative colitis. *Journal of Digestive Diseases*. 2010;**11**(6):369-375. DOI: 10.1111/j.1751-2980.2010.00462.x

[73] McIntire JJ, Umetsu SE, Akbari O, et al. Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. *Nature Immunology*. 2001;**2**(12):1109-1116. DOI: 10.1038/ni739

[74] Freeman GJ, Casasnovas JM, Umetsu DT, et al. TIM genes: A family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. *Immunological Reviews*. 2010;**235**(1):172-189. DOI: 10.1111/j.0105-2896.2010.00903.x

[75] Wolf Y, Anderson AC, Kuchroo VK. TIM3 comes of age as an inhibitory receptor. *Nature Reviews. Immunology*. 2020;**20**:173-185. DOI: 10.1038/s41577-019-0224-6

[76] Anderson AC. Tim-3: An emerging target in the cancer immunotherapy landscape. *Cancer Immunology Research*. 2014;**2**:393-398. DOI: 10.1158/2326-6066.CIR-14-0039

[77] Andrews LP, Marciscano AE, Drake CG, et al. LAG3 (CD223) as a cancer immunotherapy target. *Immunological Reviews*. 2017;**276**(1):80-96. DOI: 10.1111/imr.12519

[78] Ming Q, Celas DP, Wu C, et al. LAG3 ectodomain structure reveals functional

interfaces for ligand and antibody recognition. *Nature Immunology*. 2022;**23**(7):1031-1041. DOI: 10.1038/s41590-022-01238-7

[79] Mariuzza RA, Shahid S, Karade SS. The immune checkpoint receptor LAG3: Structure, function, and target for cancer immunotherapy. *The Journal of Biological Chemistry*. 2024;**300**(5):107241. DOI: 10.1016/j.jbc.2024.107241

[80] Nguyen LT, Ohashi PS. Clinical blockade of PD1 and LAG3-potential mechanisms of action. *Nature Reviews. Immunology*. 2015;**15**:45-56. DOI: 10.1038/nri3790

[81] Andrzejczak A, Karabon L. BTLA biology in cancer: From bench discoveries to clinical potentials. *Biomarker Research*. 2024;**12**(1):8. DOI: 10.1186/s40364-024-00556-2

[82] Garapati VP, Lefranc M-P. IMGT colliers de perles and IgSF domain standardization for T cell costimulatory activatory (CD28, ICOS) and inhibitory (CTLA4, PDCD1 and BTLA) receptors. *Developmental and Comparative Immunology*. 2007;**31**:1050-1072. DOI: 10.1016/j.dci.2007.01.008

[83] Sedy JR, Gavrieli M, Potter KG, et al. B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator. *Nature Immunology*. 2005;**6**:90-98. DOI: 10.1038/ni1144

[84] Watanabe N, Gavrieli M, Sedy JR, et al. BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. *Nature Immunology*. 2003;**4**:670-679. DOI: 10.1038/ni944

[85] Huang A, Yang XR, Chung WY, et al. Targeted therapy for hepatocellular carcinoma. *Signal Transduction*

and Targeted Therapy. 2020;5:146. DOI: 10.1038/s41392-020-00264-x

[86] Twomey JD, Zhang B. Cancer immunotherapy update: FDA-approved checkpoint inhibitors and companion diagnostics. The AAPS Journal. 2021;23:39. DOI: 10.1208/s12248-021-00574-0

[87] Sara A, Ruff SM, Noonan AM, et al. Real-world use of immunotherapy for hepatocellular carcinoma. Pragmatic and Observational Research. 2023;14:63-74

[88] Wong JSL, Kwok GGW, Tang V, et al. Ipilimumab and nivolumab/pembrolizumab in advanced hepatocellular carcinoma refractory to prior immune checkpoint inhibitors. Journal for Immunotherapy of Cancer. 2021;9:2. DOI: 0.1136/jitc-2020-001945

[89] Yau T, Park JW, Finn RS, et al. Nivolumab versus sorafenib in advanced hepatocellular carcinoma (CheckMate 459): A randomised, multicentre, open-label, phase 3 trial. The Lancet Oncology. 2022;23:77-90. DOI: 10.1016/S1470-2045(21)00604-5

[90] Zhu AX, Finn RS, Edeline J, et al. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): A non-randomised, open-label phase 2 trial. The Lancet Oncology. 2018;19:940-952. DOI: 10.1016/S1470-2045(18)30351-6

[91] Finn RS, Ryoo BY, Merle P, et al. Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: A randomized, double-blind, phase III trial. Journal of Clinical Oncology. 2020;38:193-202. DOI: 10.1200/JCO.19.01307

[92] Lee MS, Ryoo BY, Hsu CH, et al. Atezolizumab with or without bevacizumab in unresectable hepatocellular carcinoma (GO30140): An open-label, multicentre, phase 1b study. The Lancet Oncology. 2020;21:808-820. DOI: 10.1016/S1470-2045(20)30156-X

[93] Qin S, Ren Z, Meng Z, et al. Camrelizumab in patients with previously treated advanced hepatocellular carcinoma: A multicentre, open-label, parallel-group, randomised, phase 2 trial. The Lancet Oncology. 2020;21:571-580. DOI: 10.1016/S1470-2045(20)30011-5

[94] Finn RS, Qin S, Ikeda M, et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. The New England Journal of Medicine. 2020;382:1894-1905. DOI: 10.1056/NEJMoa1915745

[95] Abou-Alfa GK, Chan SL, Kudo M, et al. Phase 3 randomized, open-label, multicenter study of tremelimumab (T) and durvalumab (D) as first-line therapy in patients (pts) with unresectable hepatocellular carcinoma (uHCC): HIMALAYA. Journal of Clinical Oncology. 2022;40:379. DOI: 10.1200/JCO.2022.40.4_suppl.37

[96] Kelley RK, Sangro B, Harris W, et al. Safety, efficacy, and pharmacodynamics of tremelimumab plus durvalumab for patients with unresectable hepatocellular carcinoma: Randomized expansion of a phase I/II study. Journal of Clinical Oncology. 2021;39:2991-3001. DOI: 10.1200/JCO.20.03555

[97] Keam SJ. Tremelimumab: First approval. Drugs. 2023;83:93-102. DOI: 10.1007/s40265-022-01827-8

[98] Shen W, Chen Y, Lei P, et al. Immunotherapeutic approaches for treating hepatocellular carcinoma.

Cancers (Basel). 2022;**14**:5013.
 DOI: 10.3390/cancers14205013

[99] Meyers BM, Knox JJ, Liu DM, et al. The evolution of immune checkpoint inhibitor combinations in advanced hepatocellular carcinoma—A systematic review. *Cancer Treatment Reviews*. 2023;**118**:102584. DOI: 10.1016/j.ctrv.2023.102584

[100] Yi M, Jiao D, Qin S, et al. Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment. *Molecular Cancer*. 2019;**18**:60. DOI: 10.1186/s12943-019-0974-6

[101] Yau T, Kang YK, Kim TY, et al. Efficacy and safety of nivolumab plus ipilimumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib: The CheckMate 040 randomized clinical trial. *JAMA Oncology*. 2020;**6**:e204564. DOI: 10.1001/jamaoncol.2020.4564

[102] Duffy AG, Ulahannan SV, Makorova-Rusher O, et al. Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma. *Journal of Hepatology*. 2017;**66**:545-551. DOI: 10.1016/j.jhep.2016.10.029

[103] Finn RS, Ikeda M, Zhu AX, et al. Phase Ib study of lenvatinib plus pembrolizumab in patients with unresectable hepatocellular carcinoma. *Journal of Clinical Oncology*. 2020;**38**:2960-2970. DOI: 10.1200/JCO.20.00808

[104] Li H, Qin S, Liu Y, et al. Camrelizumab combined with FOLFOX4 regimen as first-line therapy for advanced hepatocellular carcinomas: A sub-cohort of a multicenter phase Ib/II study. *Drug Design, Development and Therapy*. 2021;**15**:1873-1882. DOI: 10.2147/DDDT.S304857

[105] Zheng X, Liu X, Lei Y, Wang G, et al. Glypican-3: A novel and promising target for the treatment of hepatocellular carcinoma. *Frontiers in Oncology*. 2022;**12**:824208. DOI: 10.3389/fonc.2022.824208

[106] Sau S, Alsaab HO, Bhise K, et al. Multifunctional nanoparticles for cancer immunotherapy: A groundbreaking approach for reprogramming malfunctioned tumor environment. *Journal of Controlled Release*. 2018;**274**:24-34. DOI: 10.1016/j.jconrel.2018.01.028

[107] Kingwell K. CAR T therapies drive into new terrain. *Nature Reviews. Drug Discovery*. 2017;**16**:301-304. DOI: 10.1038/nrd.2017.84

[108] Grosser R, Cherkassky L, Chintala N, et al. Combination immunotherapy with CAR T cells and checkpoint blockade for the treatment of solid tumors. *Cancer Cell*. 2019;**36**:471-482. DOI: 10.1016/j.ccell.2019.09.006

[109] Shi D, Shi Y, Kaseb AO, et al. Chimeric antigen receptor-glypican-3 T-cell therapy for advanced hepatocellular carcinoma: Results of phase I trials. *Clinical Cancer Research*. 2020;**26**:3979-3989. DOI: 10.1158/1078-0432.CCR-19-3259

[110] Yu M, Luo H, Fan M, et al. Development of GPC3-specific chimeric antigen receptor-engineered natural killer cells for the treatment of hepatocellular carcinoma. *Molecular Therapy*. 2018;**26**:366-378. DOI: 10.1016/j.ymthe.2017.12.012. DOI: 10.1016/j.ymthe.2017.12.012

[111] Meng F, Zhao J, Tan AT, et al. Immunotherapy of HBV-related advanced hepatocellular carcinoma with short-term HBV-specific TCR expressed T cells: Results of dose escalation, phase I trial. *Hepatology International*.

2021;**15**(6):1402-1412. DOI: 10.1007/s12072-021-10250-2

[112] Tan AT, Meng F, Jin J, et al. Immunological alterations after immunotherapy with short lived HBV-TCR T cells associates with long-term treatment response in HBV-HCC. *Hepatology Communications*. 2022;**6**(4):841-854. DOI: 10.1002/hep4.1857

[113] Kessler T, Wick W. Oncolytic virotherapy: Potentially a game-changing tumor treatment. *Cancer Cell*. 2021;**39**:753-755. DOI: 10.1038/s41467-020-18444-2

[114] Heo J, Reid T, Ruo L, et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver cancer. *Nature Medicine*. 2013;**19**:329-336. DOI: 10.1038/nm.3089

[115] Moehler M, Heo J, Lee HC, et al. Vaccinia-based oncolytic immunotherapy Pexastimogene Devacirepvec in patients with advanced hepatocellular carcinoma after sorafenib failure: A randomized multicenter phase IIb trial (TRAVERSE). *Oncoimmunology*. 2019;**8**:1615817. DOI: 10.1080/2162402X.2019.1615817

[116] Saxena M, van der Burg SH, Melief CJM, et al. Therapeutic cancer vaccines. *Nature Reviews. Cancer*. 2021;**21**:360-378. DOI: 10.1038/s41568-021-00346-0

[117] Loffler MW, Gori S, Izzo F, et al. Phase I/II multicenter trial of a novel therapeutic cancer vaccine, HepaVac-101, for hepatocellular carcinoma. *Clinical Cancer Research*. 2022;**28**:2555-2566. DOI: 10.1158/1078-0432.CCR-21-4424

[118] Rizell M, Sternby Eilard M, Andersson M, et al. Phase 1 trial with the cell-based immune primer ilixadencel, alone, and combined with sorafenib, in advanced hepatocellular carcinoma.

Frontiers in Oncology. 2019;**9**:19. DOI: 10.3389/fonc.2019.00019

[119] Lin M, Liang S, Wang X, et al. Cryoablation combined with allogenic natural killer cell immunotherapy improves the curative effect in patients with advanced hepatocellular cancer. *Oncotarget*. 2017;**8**:81967-81977. DOI: 10.18632/oncotarget.17804

[120] Ohira M, Hotta R, Tanaka Y, et al. Pilot study to determine the safety and feasibility of deceased donor liver natural killer cell infusion to liver transplant recipients with hepatocellular carcinoma. *Cancer Immunology, Immunotherapy*. 2022;**71**:589-599. DOI: 10.1007/s00262-021-03005-3

[121] Xu L, Wang J, Kim Y, et al. A randomized controlled trial on patients with or without adjuvant autologous cytokine-induced killer cells after curative resection for hepatocellular carcinoma. *Oncotargets and Therapy*. 2016;**5**:e1083671. DOI: 10.1080/2162402X.2015.1083671

[122] Lee JH, Lee JH, Lim YS, et al. Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. *Gastroenterology*. 2015;**148**:1383-1391.e6. DOI: 10.1053/j.gastro.2015.02.055

[123] Zhu AX, Abbas AR, de Galarreta MR, et al. Molecular correlates of clinical response and resistance to atezolizumab in combination with bevacizumab in advanced hepatocellular carcinoma. *Nature Medicine*. 2022;**28**:1599-1611. DOI: 10.1038/s41591-022-01868-2

Section 4

Shaping the Future: mRNA Vaccines and Oncolytic Therapies

Neoantigen mRNA Cancer Vaccines: How Deep Learning Is Advancing Design and Breaking Barriers

Saba Ismail and Khaled Barakat

Abstract

mRNA-based cancer vaccines have emerged as a transformative approach to combating cancerous cells among all cancer immunotherapy options. mRNA vaccines that encode tumor-specific antigens (TSAs) or neoantigens are responsible for a robust response to cancer. Unlike DNA vaccines, neoantigen-based mRNA vaccines are the preferred choice due to ease in scalable production, flexible neoantigen design, improved stability, structural optimization for enhanced translation efficiency, and decreased chances of genomic integration. Encapsulation of nonreplicating mRNA into a lipid nanoparticle-based delivery system further augments mRNA uptake and promotes endosomal escape. Recent advancements in machine learning and deep learning models can assist in designing solutions to the recurring problems of neoantigen mRNA vaccines by addressing the challenges such as tumor heterogeneity, neoantigen variability, the discovery of novel biomarkers, antigen presentation, and modulation of tumor microenvironment (TME) through initial experimental validations. This chapter highlights the convergence of mRNA technology with AI-optimized neoantigen prediction using machine learning and deep learning tools for effective therapeutic options.

Keywords: mRNA vaccines, cancer immunology, immunogenicity of mRNA vaccines, artificial intelligence, personalized cancer vaccines, tumor microenvironment, delivery systems

1. Introduction

Cancer immunotherapies have challenged the conventional treatment options for cancer that detect the tumor antigens and effectively eliminate the malignant cancerous cells from the host body [1]. The passive approach for neoplasm immunotherapy involves monoclonal antibodies and adoptive T-cell therapy [2]. The active cancer immunotherapy approach that harnesses the power to generate a specific, robust, long-term, and sustained immune response includes cancer vaccines [3]. Among cancer vaccines, nucleic acid-based vaccines, such as RNA cancer vaccines, have

attracted researchers due to the production of significant clinical outcomes in cancer patients without prior knowledge and restriction to the patient's human leukocyte antigens (HLAs). RNA-based cancer vaccines avoid risks like genomic integration and infection, making them safer than DNA- or viral-based platforms. The rapid, flexible design of RNA cancer vaccines enables personalized treatment targeting tumor-specific antigens [4].

The promising RNA cancer vaccines encompass RNA molecules that encode anti-tumor antigens to provoke cell-mediated and humoral immunity against the rapidly dividing cancerous cells [5]. RNA-based cancer vaccines have two principal approaches: direct vaccination using mRNA or RNA pulsed *in vitro* dendritic cells (DCs) as cancer vaccines [6]. The limitations and issues of DC cancer vaccines, including vaccine dose, ways of DC vaccine administration for robust immune response, number of antigens pulsed into DC (whether various tumor-associated antigens (TAAs) or RNA from the whole tumor), amplification, and *in vitro* RNA transcription drift, shift the focus toward mRNA cancer vaccines to achieve optimal immune response [7].

The concept of mRNA as a vaccine was introduced by Wolf et al. when they directly transfected the skeletal muscle cells with mRNA-encoding proteins luciferase and chloramphenicol acetyltransferase for their expression in mice in 1990 [8].

In 1993, research commenced on preventing tumor progression using mRNA cancer vaccines [9]. The successful induction of anti-influenza cytotoxic T lymphocytes (CTLs) with the help of mRNA-encapsulated liposomes encoding nucleoprotein of influenza into mice led to the development of mRNA vaccines encoding tumor-associated or tumor-specific antigens transfected into dendritic cells in both *in vitro* and *in vivo* experiments [7, 9]. As technological advancements prevailed, scientists eradicated the early drawbacks of mRNA cancer vaccines, such as low expression efficiency and RNA instability, with the optimized structure of mRNA, increased stability, efficient delivery methods, and lucrative clinical trials [10]. Until now, scientists have approved and used various mRNA vaccines against infectious diseases caused by the human immunodeficiency virus (HIV), rabies virus, Zika virus, encephalitis virus, and bacterium named *Mycobacterium tuberculosis* [11]. For the past few years, they have been used as protein replacement therapy for abnormal proteins such as erythropoietin, factor IX, ornithine transcarbamylase, and follistatin [12]. mRNA vaccines ushered in a new era of rapid vaccine development when, in 2020, researchers got the two mRNA-based COVID-19 vaccines by Moderna Inc. (mRNA-1273) and Pfizer-BioNTech (BNT162b2) approved in an emergency from the FDA [13]. Subsequently, the approval of other mRNA-based vaccines against SARS-CoV-2 infections produced by other companies worldwide in the next year will further stimulate the development of mRNA-based cancer vaccines [14, 15].

The development of mRNA vaccines encoding tumor-specific antigens called neoantigens incessantly faces challenges due to the limited data and insufficient computational tools for neoantigen prediction, eventually influencing vaccine efficacy against tumors. Advanced bioinformatics tools, including machine learning and deep learning, address such challenges in vaccine development to overcome the hurdles in predictive results. They provide accurate results through an extensive evaluation of cancer of input data from immunological profiles. Integration of AI, machine, and deep learning tools, such as support vector machines (SVMs), random forest plots, convolutional neural networks (CNNs), and graph neural networks (GNNs), is crucial for refining results of mRNA secondary prediction, neoantigen prediction, novel biomarker discovery, optimization of lipid nanoparticle (LNP)-encapsulated mRNA vaccine, and

immunogenicity potential of vaccine candidates. This chapter describes the basics of mRNA cancer vaccines, the challenges involved in vaccine design, and the role of deep learning in addressing these challenges. The study aims to address the mentioned challenges in neoantigen cancer design through the role of AI-driven insights and advanced deep learning models to design and develop personalized mRNA cancer vaccines.

2. Classification of mRNA cancer vaccines

2.1 Based on encoding antigens

Selecting an antigen with a robust yet controllable T-cell immune response with high specificity against tumors is one of the primary steps in developing an mRNA vaccine [16]. Since the new technologies involve the cost-effective production of tumor sequences of *in vitro* transcribed mRNA, each mRNA cancer vaccine encoding different tumor antigens has promising immune responses after identifying specific tumor target sites [17]. There are three types of tumor antigens that mRNA vaccines can encode: (a) Tumor-specific antigens (TSAs) or neoantigens: They are overexpressed on cancerous cells due to non-synonymous mutations in the neoplastic cell genome or aberrant posttranslational modifications (PTMs) [18]. MHC class I molecules represent them on the tumor cell surface, making them highly specific for tumors with low central tolerance levels, strong immunogenic potential, and low tumor regression rate [19]. They are homogeneously produced by tumor cells having a mutational load greater than 150 non-synonymous mutations. Each tumor can produce thousands of TSAs, as evidenced by exon sequencing analysis of 13 tumors of different cancers [20]. Various studies reported an increased level of cytotoxic T lymphocytes (CTLA4), programmed cell death (PD-1), and CD8A associated with high levels of immunogenic neoantigens, which eventually leads to a high survival rate in endometrial cancer, as well as RNA sequence analysis from the Cancer Genome Atlas [21]. Machine learning, next-generation exon sequencing, and automated screening have enabled TSA prediction by identifying somatic mutations and abnormal transcription and translation processes [22]. (b) Tumor-associated antigens (TAAs): They are generally overexpressed on neoplastic cells but are also expressed at a low level in normal cells, making them unique in tumors. TAAs comprise carcinoembryonic and tissue differentiation antigens with low immunogenic potential, tumor specificity, and central tolerance levels. They are non-mutated self-antigens, which cause poor immune response in cancer immunotherapy. TAAs on normal cells also cause collateral damage [23]. Currently, mRNA cancer vaccines targeting multiple TAAs are combined with vectors and adjuvants to stimulate a robust anti-tumor response in the tumor microenvironment in the clinical setting [24]. (c) Viral Antigens: mRNA cancer vaccines encoding viral antigens are making waves in cancer immunotherapy due to their remarkable therapeutic response in various cancer types associated with viral infections such as HIV, human papillomavirus (HPV), hepatitis B, and Epstein-Barr virus [25]. Current research demonstrated mRNA cancer vaccines' strong immunogenic and anti-cancerous effects targeting HPV in animal trials [26].

2.2 Based on the nature of mRNA molecule

mRNA cancer vaccines are also classified into nonreplicating or conventional mRNA, self-amplifying (saRNA), and trans-amplifying mRNA cancer vaccines

(taRNA) based on the replicating nature of messenger RNA [27]. The traditional mRNA vaccines consist of untranslated regions (UTRs) of mRNA alongside the encoded antigens [28]. The benefits of conventional mRNA vaccines include specific immune response, small RNA size, and simple structure [29]. Contrarily, self-amplifying mRNA vaccines can tackle infections with maximum efficacy and minimal effects. They contain the self-replicating single-stranded RNA (+) (ssRNA). They are also known as virus-inspired vaccines, as the viruses contain the unique replicating RNA that exploits host nucleotides and ribosomes to form the replicating machinery for the virus [30]. saRNA vaccines own the alphavirus genome encoding viral non-structural proteins (nsPs), which replace the preferred antigen to elicit an immune response. It allows saRNA vaccines to produce multiple copies of RNA; consequently, a large yield of vaccine antigens is expressed [31]. A high antigen expression level can be achieved at relatively low doses of saRNA vaccines [32]. However, they are of lengthy sequences (9–12 kb), making their production more arduous on an industrial level than a conventional mRNA vaccine [33].

3. Components of mRNA cancer vaccines

Generally, the mRNA cancer vaccine comprises antigens, adjuvants, and targeted advanced drug delivery systems [34]. The tumor antigen should be highly tumor-specific to elicit a potent and sustained cellular and humoral immune response by the mRNA cancer vaccine [35]. The other fundamental immune component is adjuvant, which should synergistically affect neoantigen-related immune responses [36]. Furthermore, the delivery system and vectors are required to deliver mRNA molecules because of the anionic, easily degradable, and low penetration nature of mRNA molecules [37]. Subsequently, the mRNA sequence is optimized by modifying its components to make the mRNA cancer vaccine more efficient [38]. However, the structural integrity of the mRNA sequence should be preserved for its stability and expression capacity in the host (**Figure 1**) [39].

mRNA in a cancer vaccine is a negatively charged and single-stranded molecule generally composed of an mRNA sequence, which is made up of (i) a 5' cap (5 prime caps), (ii) a 5' untranslated region (5 prime UTR), (iii) an open reading frame (ORF), (iv) a 3' untranslated region (3 prime UTR), and (v) a poly (A) tail [40]. The mRNA sequence is modified to encode the tumor antigens translated into the targeted protein. The *in vitro* transcription (IVT) process for mRNA synthesis has been much more developed [41]. The raw products obtained from the *in vitro* transcription process of linear plasmid DNA are the required mRNA, untargeted or nonspecific mRNA, oligonucleotides, nucleotides, and various proteins [42]. Targeted mRNA is then purified and extracted from the product mixture by precipitation methods to remove the impurities and high-performance liquid chromatographic (HPLC) techniques to separate it from the rest of the components [43]. The five prime cap is one of the essential structural elements of the mRNA sequence. The 5' cap comprises 7-methyl guanosine (7mG) attached to the 5' end of mRNA (m7GpppN), naturally occurring in eukaryotic organisms [44]. The cap is attached to the first nucleotide of the five prime ends of RNA *via* a tripolyphosphate connection, eliminating the phosphate-free groups, thus enhancing mRNA's stability. It also plays a vital role in mRNA maturation by regulating pre-mRNA splicing and export from nuclei, protecting the RNA from exonuclease degradation, and initiating its translation [45]. In addition to maturing the pre-mRNA, the m7GpppN cap helps recognize the host

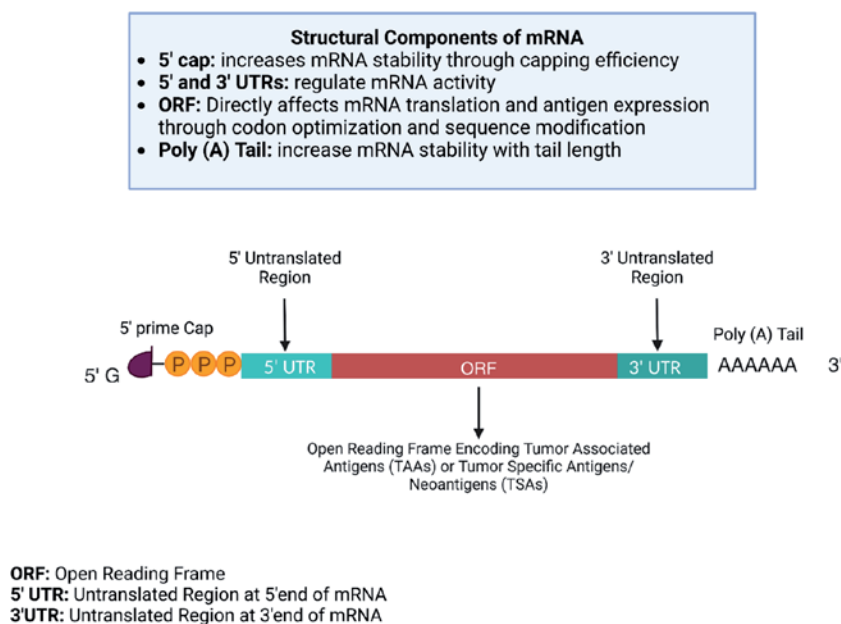


Figure 1.
Structural components of mRNA-encoding tumor antigens. mRNA has a 5' cap, 5' and 3' untranslated regions (UTRs), an open reading frame (ORF), and a poly(A) tail at 3' end.

self mRNA molecule and its binding affinity for the eIF4F heterodimeric complex to initiate the translation process [46]. *In vitro* capping of mRNA is done in two ways. The first approach involves the addition of cap analogs, whether regular traditional m7GpppN caps or anti-reverse cap analogs (ARCA), together with the *in vitro* transcription process. The other approach for mRNA capping is through an enzymatic reaction following the *in vitro* transcription (IVT) process [47].

The conventional capping of IVT mRNA with m7GpppN made the isomers of mRNA as cap analogs that can bind to the mRNA sequence reversibly. The formation of RNA isomers leads to decreased mRNA translation, making mRNA vaccines less efficient [48]. Five prime cap anti-reverse analogs were introduced by modifying the carbon number 2 or 3, ensuring the reaction of the cap methyl group with the hydroxyl group at the binding site during the transcription process to avoid cap reversible addition in mRNA [49].

The *in vivo* mRNA expression and mRNA stability are regulated by the 5' and 3' UTR. The regions contain codons that are not translated by translation factors in the host. Studies have shown that both regions are vital in converting mRNA into its secondary structures, effective recognition, and interaction by ribosomes, as well as translation factors and components [50].

5' UTRs directly influence the rate of translational efficacy and stability, as they determine the translation of the subsequent open ORF sequences. To ensure accurate translation, the 5' UTRs should avoid genome sequences identical to upstream ORF regions or start codons. The optimization of 5' UTRs by adding specific sequences such as GCC-(A/G)-CCAUGG revealed a targeted start of the translation process. Researchers reported that avoiding lengthy 5' UTRs and highly stable mRNA secondary structures and replacing them with short and loose sequences of 5' UTRs *in vitro* transcribed mRNA are more favorable for mRNA translation [51].

3' UTR is present at the three prime ends of mRNA. The decay of mRNA and the expression level of mRNA *in vivo* are also significantly associated with the modification of 3' UTR [50]. The most used 3' UTR sequence originated from human alpha and beta globin mRNA in IVT mRNA [52]. Though 3' UTR contains complete AU and GU pair sequences as unstable factors, preventing such sequences and incorporating the stable elements in 3' and 5' UTRs directly influence the rate of translational efficacy and stability, as they determine the translation of the subsequent ORF sequences. 3' UTR significantly improves the mRNA stability and decay half-life. The attachment of two 3' UTRs containing stable components can lucratively increase the efficacy of mRNA translation [53].

The crucial component of the mRNA sequence is an ORF due to the presence of translatable codons. The ORF contains readily translated codons and rare codons [54]. Therefore, researchers can employ different codon optimization strategies to increase the yield and amplification of mRNA and proteins [55]. The introduction of highly expressed genes in linear plasmid DNA replacement of rare codons with frequent codons of identical sequences, which have a higher abundance of tRNA, boosts the translation process of mRNA [56]. ORF should not have the upstream start codons before the actual codons [57]. The translation rate is crucial to producing a high-quality protein with an optimized and effective folding structure [58]. The presence of rare or uncommon codons in an open reading frame ensures a delayed translation process to yield highly folded quality proteins [59]. Hence, a high expression rate can sometimes be detrimental, as some proteins require delayed translation of the ORF [57]. Some antigens demand slower or higher translation rates; depending on that, scientists apply different optimization strategies for ORF codons [60].

Poly (A) tail is another critical element that affects RNA stability, increases the *in vivo* half-life, enhances translation, and prevents mRNA from degradation by RNA exonucleases [61]. The terminal part of mRNA comprises 250 nucleotides in healthy mammalian cells. Its length gradually decreases over time within the cytoplasm. However, most mRNA vaccines should contain mRNA that has a poly (A) tail 100 nucleotides long. Different cells have different lengths of poly (A) tail structures in their mRNA [62]. It was observed that well-translated mRNA usually has shorter poly (A) tail sequences in dendritic cells, while T cells' mRNA has lengthy poly (A) tail sequences for optimal and efficient translation [62]. Therefore, adjustments should be made to optimize the poly (A) tail length and mRNA translation. The tail has poly (A) binding proteins (PABP) that link with the 5' cap with the aid of eIF4E and eIF4G (initiation factors of translation), which helps mRNA to remain enclosed in a loop-like structure [63]. This adjoining ultimately assists the poly (A) tail in maintaining stability and mRNA translation efficiency [64].

4. Delivery system for mRNA cancer vaccines

Earlier experiments demonstrated that naked RNA can be inserted into the host to reach the target site subcutaneously, intramuscularly, or intradermally. Nevertheless, large size (approximately 106 Dalton), negative charge, longer half-life (7 hours), and easy degradation by RNA exonucleases in dermal and other tissues hinder the systemic absorption and uptake of naked RNA without any delivery system. Subsequently, the translation efficacy and tumor antigen expression become insignificant. Naked mRNA also faces the problem of permeation in the skin when injected *via* a subcutaneous route [65]. Though various techniques such as microneedling,

iontophoresis, jet injection, sonophoresis, and electroporation have been developed, the administration of mRNA without any adjuvant or delivery system still has unavoidable problems. Therefore, many modalities for delivering mRNA cancer vaccines in the host are being designed. The mRNA can be mediated by viral vectors and delivery systems made of lipids and polymers [66].

Viral vector-based mRNA vaccine delivery involves delivering viruses with genetic modifications. Typical examples are flavivirus, alphavirus, and picornavirus [67]. However, the significant drawbacks linked with viral vectors are cytopathogenicity, host rejection response, and genetic integration into the host genome, which can go unnoticed [68]. Hence, lipid-based and polymer-based delivery systems are needed for mRNA cancer vaccines. With successful vaccine development for COVID-19, LNPs comprising cationic lipids encapsulating mRNA vaccines are making waves in cancer immunotherapy globally [69]. The lipid delivery system includes mRNA cancer vaccine delivery in LNPs, liposomes, solid-lipid nanoparticles, nano lipid carriers, lipoplexes, and cationic nano-emulsions of variable sizes ranging from 80 to 200 nm [70]. Endocytosis can move LNPs and other forms into cells [71]. The elements of the lipid system can hold the polyanionic RNA molecule and enclose it into a 100 nm particle size by aggregating it. Each component of the lipid system plays a significant role in creating an efficient delivery system; the cationic lipid assists in releasing the RNA through ionization into the cytoplasm, polyethylene glycol (PEG) prevents the LNP-mRNA complex from degradation and increases the half-life of the complex, cholesterol enhances the structural compatibility and stability of LNPs enclosing the mRNA vaccine, and phospholipids allow the nanoparticle complex to make a lipid bilayer structure for its smooth transfer across the lipid cell membrane [72]. The frequently used cationic lipids to deliver mRNA are DOTAP and DOTMA [48, 73]. The different administration routes of LNPs can induce different immune responses. The high yield of mRNA is achieved through IV injections of LNPs, while local effects can be mediated through dermal or topical delivery routes, which are subcutaneous, intradermal, and intramuscular in metastases treatment [74]. Currently, LNPs having mRNA targeting various TSAs (neoantigens) as monotherapy or combined therapy with a monoclonal antibody named pembrolizumab are being investigated in the treatment of cutaneous melanoma, solid tumors, gastrointestinal cancer, pancreatic cancer, non-small cell lung cancer (NSCLC), and colorectal cancer [75]. The polymer delivery system involves the encapsulation of mRNA between the strong binding of cationic polymers and anionic nucleic acids such as RNA [76]. The bonding prevents the breakdown of RNA through ribonucleases, thus playing a vital role in mRNA translation efficiency and stability. Examples of polymers include polyesters such as poly lactic glycolic acid (PLGA), PEI, dendritic polymers, and polyamino acids [77]. The polymers are beneficial in terms of extended half-life, long shelf life, aggregation of nanostructures in water conditions, and lyophilization. The polymer is combined with a lipid delivery system for tunable targeting of tumor antigens encoded by the mRNA cancer vaccine in various cancers [78]. However, the polymers used in the delivery systems are potentially more toxic than the LNP complex. They do not get engulfed quickly by cytoplasm, thus having a lower transfection rate [79]. A lipopolyplex is a hybrid delivery system in which a lipid shell encapsulates a complex of anionic mRNA with a cationic lipid molecule [80]. It tends to avoid agglomeration more than conventional lipid systems. It provides remarkable stability and efficiency, less toxicity, increased genome transfer, and facilitated endocytosis in antigen-presenting cells (APCs) [81]. A lipoplex vaccine named BNT111 targeting four TSAs is evaluated for its efficacy in advanced melanoma [82]. The same lipoplex vaccine

is also assessed as monotherapy or combined therapy with anti-PD-1 cemiplimab against refractory terminal melanoma in clinical trials [83]. Lipoplex-encoding tumor-specific antigens known as BNT122 are also evaluated for their efficacy against solid tumor diagnoses and treatment in different phases [84].

5. Mechanism of mRNA vaccines

5.1 mRNA release and antigen presentation

Generally, mRNA cancer vaccines activate the immune system through the same pathways as mRNA vaccines work against infections. A cancer vaccine encompasses adjuvant and immunogenic components. The immunogenic or antigenic component encodes the protein, and the adjuvant stimulates an innate immune response and T-cell activation [85]. In mRNA cancer vaccines, the delivery system, such as ionizable LNPs, encapsulates mRNA, which can function as both an immunogen and an adjuvant. The LNP and mRNA complexes are highly effective adjuvants. When LNPs with mRNA-encoding tumor antigens enter the human body, two types of immune responses are produced: (i) an innate immune response and (ii) an adaptive immune response [86]. The ionizable lipid molecule, a critical adjuvant in mRNA cancer vaccines, allows the infusion with the host cell cytoplasm and delivers the mRNA through endocytosis. It also promotes mRNA's cellular uptake and escape from an endosome. Other than ionizable lipids, mannosylated lipids encapsulated mRNA, causing mRNA to accumulate into APCs rapidly and, consequently, increasing mRNA uptake by APCs [87]. A strong anticancer response is initiated when antigen-presenting cells mature and show increased antigen-presentation sites on their surfaces. The toll-like receptor 4 (TLR-4) fuses with LNPs, thus mediating internalization and endocytosis through fusion with endosomes [88].

Both types of mRNA cancer vaccines encode TAA and TSA, which follow the exact core immune activation mechanism [89]. Once mRNA enters APCs, it undergoes endocytosis by fusing with early and late endosomes. Some endosomes are recycled and removed from the cells by exocytosis [90]. The other route is mRNA degradation, when mRNA is trafficked into lysosomes [90]. The small amount of mRNA from LNP can escape the heavy traffic of endosomes to stimulate both types of immunity [91]. mRNA undergoes translation to make antigens, which degrade into smaller peptide fragments. The peptides bind to major histocompatibility molecules, which appear on the outer surface of APCs. The nonimmune nucleated cells uptake mRNA and attach the resulting peptide fragment after antigen expression through MHC I molecules. However, APCs can induce an immune response by presenting antigens through MHC I and II molecules. Off-targeting, along with immunological tolerance, is a significant problem in the case of mRNA vaccines encoding TAAs, which can cause antigen expression across several types of tumors. However, tumor-specific antigen-coded mRNA vaccines, such as those targeting KRAS mutations, play a significant role in tumor specificity with less chance of producing off-target effects. KRAS-mutated antigens break into smaller peptide fragments, bind to MHC I molecules, and activate cytotoxic T lymphocytes (CTLs) and memory T cells [92].

In mRNA cancer vaccines, adjuvants also play a critical role in the rate and amount of antigen expression by initiating mRNA translation and releasing signal molecules [93]. For example, enrichment of mRNA with modified nucleotides such as methylpseudouridines and codon optimization in an open reading frame makes efficient

mRNA translation paired with boosted antigen presentation in cancer immunotherapy. Similarly, adjuvants assist in antigen presentation to dendritic cells by stimulating TLR and interferon (IFN) gene pathways through MYD88, nuclear factor kappa B (NF- κ B), and cyclic GMP-AMP synthase (cGAS) to elicit adaptive immunity [94].

5.2 Innate and adaptive immunity

A complex cascade of intracellular pathways stimulates innate immunity. The mRNA acts as an exogenous motif or pathogen-associated molecular pattern (PAMP) in the human body when it utilizes the host ribosomes to make the target antigenic proteins [66]. The pattern recognition receptors (PRRs), including retinoic acid-inducible gene-I-like (RIG-I-like) receptors (RLRs), RNA-dependent protein kinase receptors (PKRs), nucleotide oligomerization domain (NOD)-like receptors (NLRs), ZBP1, and oligoadenylate synthetase (OAS), are particularly expressed in APCs, including macrophages, DCs, and B lymphocytes, and are responsible for initiating and regulating the immune signaling pathway led by chemokines and pro-inflammatory cytokines [66, 95].

TLR 7 and 8 receptors, which reside on endosomal surfaces other than PRRs, mediate the activation of transcription factors. These factors move into the nucleus to kickstart the translation and expression process of interferon type I, III, and pro-inflammatory cytokines [96]. RLRs initiate the same cascade, as some receptors reside in the nucleus beside the cytoplasm. The activation of PRRs and TLRs significantly increases the functional capacity of dendritic cells, thus causing enhanced maturation and activation of T cells. This recognition further stimulates the release of interferon alpha and gamma (IFN- α , γ), interleukin-12 (IL-12), and tumor necrosis factor- α (TNF- α) (Figure 2) [97].

When mRNA escapes the endosomes, it undergoes translation and is processed into either type of tumor antigen. mRNA goes under degradation afterwards, which is carried into two pathways. The breakdown of mRNA by proteasomes produces intracellular peptide fragments, which combine with the MHC I to form a complex on APCs. Subsequently, the peptide-MHC I complexes are identified by CTLs, causing them to differentiate and mature, thus mediating the direct killing of cancerous cells by CTLs. The secreted antigens undergo endocytosis by lysosomes, joining with MHC II molecules to form complexes, causing differentiation and maturation of CD4+ T lymphocytes. The lymphocytes then stimulate the B lymphocytes to produce the corresponding antibodies, eventually destroying the tumor cells. mRNA vaccine efficiency also depends on generating memory B and T cells through tumor antigen expression to prevent metastasis or tumor recurrence relapse [98]. The administration of BNT162b, following the second dose in leukemia patients, resulted in persistent activation of T memory cells and CD8+ cells. Once innate immunity is activated, IFN-I impairs mRNA translation and accelerates the adaptive immune response development. After draining into lymphatic nodes, the mRNA vaccine molecules reached B cells, immature T cells, and monocytes [99]. B cells recognize the mRNA and undergo genetic mutations to produce diverse and specific immunoglobulins against the antigens [100].

On the other hand, naïve T cells mature into follicular T-cells (T_{fh}) when they reach secondary lymphatic organs known as germinal centers [101]. The mutated B-cells, called follicular B cells, then interact with the follicular T-cells to undergo a further process of robust cellular division and hypermutation in the germinal center. Additionally, this interaction assists the selection and survival of high-affinity B cells

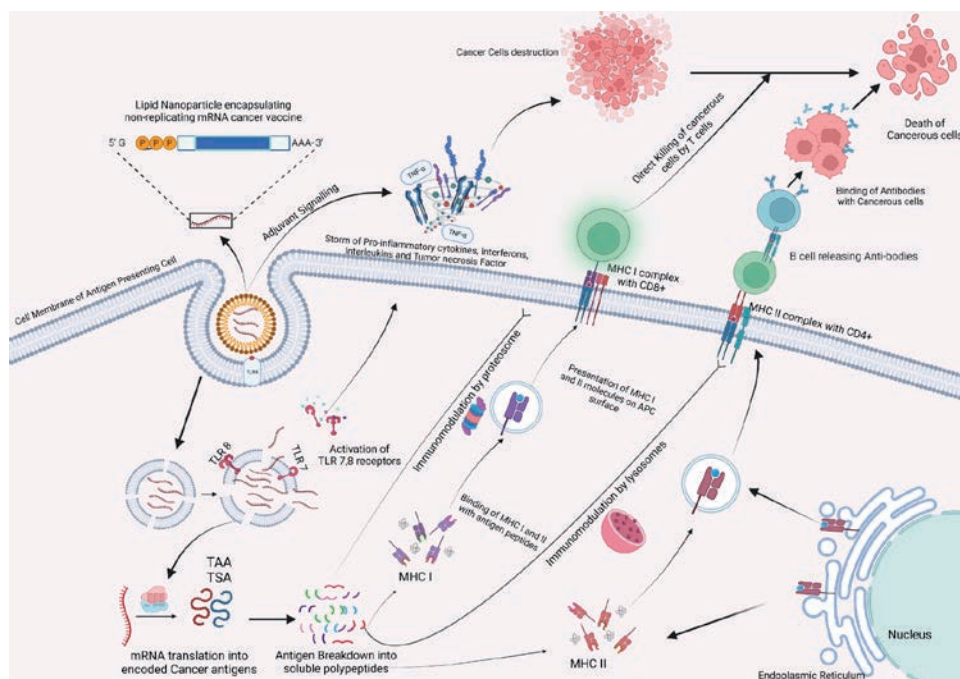


Figure 2.

Immune mechanisms of mRNA cancer vaccine. mRNA is endocytosed by TLR 4 receptor and recognized by TLR 7 and 8 receptors to produce cytokines, interferons, interleukins, and TNF- α . mRNA translates into TAAs or TSAs, which degrade into peptide fragments by proteasomes or lysosomes to bind with MHC I and MHC II molecules on the cell surface. MHC I and II complexes induce cytotoxic T lymphocyte-mediated killing of cancerous cells using CD8+ and CD4+ pathways, respectively.

from apoptosis. The critical evolutionary step differentiates B cells into high-affinity, long-lasting plasma cells (LLPCs) and germinal center long-term memory B-cells (MBCs). The plasma cells secrete two types of antibodies: the ones that will fight the immunogen and the neutralizing antibodies that will sterilize the immunity [102]. Contrarily, the MBCs generate a burst of high-affinity B cells after exposure to exogenous molecules [103]. mRNA vaccines also provoke class switching of antibodies, primarily of immunoglobulin G isotypes, ultimately leading to enhanced stimulation of defensive complement proteins and opsonization by antibodies or plasma proteins against neoplastic cells [104].

6. Benefits of mRNA cancer vaccines

mRNA cancer vaccines are drawing attention as a novel approach that utilizes genetic information to direct cells to express tumor antigens and trigger defensive cells to destroy the tumor cells [102]. mRNA molecule, the smallest genetic molecule, is safe, well-tolerated, noninfectious, and non-integrational, and it potentially encodes any tumor antigen. It is highly flexible and allows the smooth modification of the final product after the translation process [105]. Many approaches are available that modulate the immunogenic potential of mRNA, resulting in a safe immune response against tumors. Novel drug delivery approaches are the desired vehicles to safely carry and transfer the mRNA into cells, saving it from RNA exonucleases and

immediate degradation [106]. Furthermore, the manufacturing process of mRNA, particularly the *in vitro* transcribed process, is rapid, economical, and scalable. The supportive results from ongoing preclinical and clinical trials of mRNA cancer vaccines have shown their potential against several cancers, such as breast, melanoma, and prostate [107].

7. Examples of mRNA cancer vaccines

Since the revolutionary breakthrough of COVID-19 vaccines, the FDA has approved two mRNA vaccines that can be used prophylactically in cancers associated with viral infections due to their remarkable efficacy in preventing the chances of tumor cell growth [108]. The first vaccine is designed to cure HPV infections and prevent the associated cervical cancer. The other vaccine treats hepatitis B and can be used to prevent the likelihood of associated liver cancer [106, 109].

8. Challenges of mRNA cancer vaccines

Based on an extensive literature survey, the following drawbacks are considered highly crucial in order to make mRNA-based cancer vaccines.

8.1 mRNA stability issues

The relatively more straightforward and more efficient manufacturing process of mRNA produces mRNA in less time. Nevertheless, the final product is highly susceptible to instability due to extracellular RNA nucleases and high temperatures during storage and transportation. The storage, transportation, and administration conditions for mRNA delivery are super cold chain temperatures at -20 or -70°C and a sterile RNA degradative enzyme-free environment [110]. Also, mRNA hydrolyzes at a pH higher than 5 [111]. Therefore, RNA cancer vaccines must be stored in a slightly acidic medium. Apart from *in vitro* stability, the inherent *in vivo* stability of mRNA is also a significant challenge in preserving its integrity while reaching immune and nonimmune cells safely. The physicochemical properties of mRNA majorly affect the stability of the mRNA molecule. The physical degradation of mRNA involves the natural tendency of mRNA to form agglomerates, leading to compromised translation [112]. Chemical degradation involves the breaking down of mRNA by RNA 5' exonucleases, endonucleases, acids, polymerases, helicases, ribozymes, and chaperones [113]. The current modified approaches to stabilize mRNA structure are capping, optimization of the 5' and 3' UTRs, optimization of codons in the translation region of mRNA, enrichment of GC content, and addition of a poly(A) tail [114]. However, these approaches may potentially impact immunological recognition [115]. Furthermore, the optimum and stabilized formulation of an mRNA cancer vaccine should comprise sodium phosphate, sucrose, adjuvants, and encapsulation into LNPs [116].

8.2 mRNA delivery issues

Due to poor *in vivo* cellular absorption and deterioration by extracellular nucleases, successfully delivering mRNA into cells remains a significant challenge [117].

The stability and efficacy of the delivery system holding mRNA are paramount. It employs the successful delivery of mRNA to immune-stimulating cells. The malfunctioned delivery system does not effectively deliver the mRNA to target sites [118]. Self-amplifying and circular mRNA technologies are novel approaches to address the limitations of conventional nonreplicating mRNA. All forms of mRNA, whether nonreplicating, circular, or replicating, are not safe from degradative enzymes [119]. Therefore, a delivery system such as LNPs can enhance mRNA stability, distribution, and safe transportation to target tumor tissues. LNPs facilitate the targeted reach of mRNA to antigen-presenting cells and release mRNA intracellularly without any degradation. Among LNPs, ionizable lipids are preferred over other non-ionizable lipid components due to their potential for being relatively safe, carrying a neutral charge in blood after administration except when entering cells [120]. However, they still can potentially cause unintended inflammatory reactions [119].

8.3 Overcoming the immunosuppressive tumor microenvironment (TME)

The immunosuppressive milieu that many cancer types produce reduces the effectiveness of mRNA vaccines by preventing T-cell activation and antigen presentation in the TME [121]. The term tumor microenvironment came into the spotlight in the late 1800s, and researchers investigated the significant impact of TME on the aggressive growth of tumors and its circumvention by immune cells [122]. The extracellular matrix (ECM) and signaling molecules around tumor cells encumber the core functions and overall activation of natural killer cells and T-cells, obstructing the infiltration of T-cells in cancerous cells [123]. Hence, it indirectly reduces mRNA cancer vaccine efficacy [124]. A combination of mRNA with checkpoint inhibitors is being investigated to combat this problem. This combination attacks the TME and releases all the hindrances of T-cells reaching the tumor, reviving the efficacy of mRNA translation [125]. Adoptive T-cell therapy is another powerful approach to combining mRNA with a patient's tumor T-cells and its modification to encode mRNA with the targeted patient's cancer antigens [126].

8.4 Inefficient antigen presentation

APCs must effectively digest and deliver the encoded antigen for an mRNA vaccination to trigger an immune response through T-cell sensing [127]. Antigen presentation and processing (APP) pathway is directly responsible for the interaction between tumor and immune cells [128]. T cell recognition of APCs depends on their expression of a spectrum of peptides bound to MHC I and MHC II molecules. Although nonimmune cells present only the MHC II complex on their surface to be detected by T lymphocytes, the professional APCs present MHC I and II on their surfaces to activate naïve or follicular T-cells. CD8⁺ cells only recognize the MHC I complex to destroy the cancerous cells [129]. The amyloid precursor protein (APP) pathway is a complex series of processes, initiating from the breakdown of intracellular tumor-encoding antigens or defective proteins by proteasomes or lysozymes cytoplasm, triggering the release and exposure of inflammatory cytokines to cells, resulting in boosted antigen presentation by converting normal proteasomes into immunoproteasomes. Subsequently, the conversion leads to a transition into MHC I stock in cells, specificity of proteasomal cleavage, and ligand production for MHC I [130]. If they survive further degradation, the generated peptides relocate to the endoplasmic reticulum (ER) through the transporter antigen process (TAP). This process detects peptides with

9–16 amino acid residues and transports them to the ER, where they get assembled on the MHC I molecule on its groove site through a peptide loading complex (PLC). The groove site of the MHC I molecule has a space for 8–11 amino acid residues [131]. PLC and other proteins select the suitable antigen epitope and validate the quality of peptide and MHC I stable binding [132]. The Golgi apparatus transports the formed stable complex to the cell surface to be sensed by T-cells [131].

The aberrant and abnormal change in tumor cells' antigen-processing machinery (APM) resulted in immune evasion and cancer progression. The defective APM causes alteration of peptide and MHC I repertoire and downregulates the expression of MHC I molecule on the cell surface [133]. Several tumor types also reported abnormalities and alterations in different steps of the APP pathway, which leads to an unstable MHC I complex, downregulation of proteins involved in antigen editing, and MHC I assembly with suitable peptides, resulting in an ineffective immune response and increased resistance to mRNA cancer vaccine therapy alone or in combination with immune checkpoint inhibitors [134]. In conclusion, the regular and effective APM and APP pathways in tumors should remain intact for efficient mRNA translation and a robust immune response [135].

8.5 Biomarker identification

Conventional anticancer therapies rely on targeting and eradicating cancerous cells, and radiotherapy directly measures the destructive response. However, mRNA cancer vaccines indirectly generate an immune response through translation into encoded tumor antigens, which radiotherapy does not detect easily [136]. Furthermore, the mRNA cancer vaccine's immunogenic potential and efficacy as a monotherapy are considerably significant in low-burden tumors. The radiographic evaluation is not noticeable with mRNA cancer vaccines due to the above reasons in the terminal stages of cancer [137]. Therefore, quantifying the immune response generated by mRNA cancer vaccines is possible only by using established biomarkers to examine the efficacy of vaccines. It is a significant challenge to identify and devise such biomarkers that determine the vaccine doses that produce optimal response and prevent cancer recurrence [138]. Multiple pieces of research have reported the use of different biomarkers, such as chemokines, flow cytometry analysis, enzyme-linked immunospot assay (ELISPOT), tetramer analysis, single-cell RNA sequencing technology (scRNAseq), and circulating tumor DNA (ctDNA) test, which have been previously established to evaluate the immune response to vaccines. However, various limitations must be rectified to measure the exact response [138]. Therefore, an hour is needed to identify and establish novel biomarkers to check the optimal doses and select effective mRNA cancer vaccines to surpass initial clinical trials for personalized cancer immunotherapy in the late stages of disease [136, 137]. By identifying biomarkers that can forecast immunotherapeutic response, AI-driven clinical and genetic data analysis improves personalized treatment strategies [139].

8.6 Tumor heterogeneity and neoantigen variability

The tumor heterogeneity significantly influences vaccine efficacy. Since tumors are highly heterogeneous, distinct cancer cells may express various antigens within the same tumor and between individuals [140]. There are two types of heterogeneities in tumors: the variation in tumor subclones, called neoantigen variability or spatial heterogeneity, and the evolution of the tumor genome with the advancement of the

cancer stage. This diversity makes universal neoantigens appropriate for vaccine development more obscure [140]. The tumors with high temporal heterogeneity also have high cancer resistance rates to immunotherapy. Conversely, the neoantigen variability is due to the presence or absence of neoantigens in different subclones within the same tumor, resulting in higher cancer progression rates and resistance [141]. The small sampling from tumors has limited genomic data to encode for each type of neoantigen, making the efficacy of mRNA cancer vaccines on heterogeneous tumors more challenging and uncertain [142]. Sampling neoplastic cells from different tumor regions can be a promising solution for neoantigen variability. The temporal heterogeneity can be reduced by careful monitoring and adjusting the treatment options available. However, if these strategies were employed to combat temporal heterogeneity and neoantigen variability, the vaccine design, administration, cost, and clinical trials would be more complex and unfathomable. The artificial intelligence tools and next-generation sequencing (NGS) computationally evaluate various heterogeneity parameters to predict the neoantigens based on tumor genomic profile [139].

9. Challenges of mRNA cancer vaccine development and role of machine learning and deep learning approaches to address them

Recent research in immunotherapy and immunoinformatics has illustrated striking revelations about improvements through an amalgamation of deep learning and machine learning algorithms. These approaches can potentially provide robust, efficient, and scalable solutions.

9.1 Enhanced vaccine design

Machine learning and deep learning optimized tools can improve mRNA sequences regarding immunogenicity, efficient translation, and immunogenicity due to insufficient knowledge of forecasting which of the four RNA nucleotides make the base pairs in secondary structures. For instance, these techniques can conveniently predict RNA secondary structures, codon optimization, and 3D modeling. They have been proven instrumental in forecasting mRNA sequences and structure prediction to generate neoantigens with high affinity. Conventionally, AI models are categorized into three categories for structure prediction: nearest neighbor models, probabilistic generative models, and deep learning models [143].

9.1.1 Conventional models

The traditional models frequently used for predicting secondary structures are nearest neighbor models [144]. They can forecast the structure based on loop structures by closing base pairs in mRNA. The energy parameters characterized by the components in primary loop structures of RNA are evaluated through wet-lab experiments, thermodynamics-based models, and machine learning models [145]. Models that employ free energy parameter values data directly obtained from lab experiments are RNA structure [146], RNAfold present in the Vienna RNA package [147], UNAFold, or Mfold [148]. These thermodynamic-based models predict the secondary structure through Turner's free energy parameters [149], comprising up to 12,700 different energy parameters. The vast majority of RNA secondary structure prediction technologies, including RNAfold, function under the assumptions

of thermodynamic equilibrium, which might not accurately represent the dynamic folding of RNA within cells. Lorenz et al. recognized that computationally predicted static structures are not the same as real-time RNA folding *in vivo* [147]. Because AI-enhanced structure predictors are trained on existing RNA sequences, they may not accurately predict novel RNA structures. Sato et al. emphasized that RNA structural deep learning models lack high-quality training data [150].

Another approach to determining free energy parameters in RNA is machine learning. Examples of the nearest neighbor models utilizing machine learning through training of large datasets of RNA base pair sequences to predict the secondary structures are Contextfold [151] and CONTRAfold [152]. Although these models provide detailed knowledge of free energy parameter values, machine learning involves accurate parameterization with remarkable accuracy of relatively fewer Turner's free energy parameters. Contextfold, also known as the fine-grained secondary structure of RNA, can train up to 2,00,000 energy parameters [151]. Meanwhile, CONTRAfold employs conditional log-linear models (CLLMs), capable of training 300 free energy parameters with machine learning techniques [152]. The limitation of these models is an increased risk of overfitting the data, which is not observed for unknown data [153].

Probabilistic generative models such as Pfold employ context-free grammars known as stochastic context-free grammars (SCFGs) to determine the RNA secondary structure [154]. TORNADO is another advanced tool that combines context-free grammar with Turner's energy parameters to infer results comparable to nearest neighbor models [155]. However, the level of computational complexity in these models and the limited scope of determining complex features of RNA secondary structure make them impracticable in the structure prediction of RNA [156].

Hybrid models incorporating both of the above approaches are SimFold [157], MXfold [158], and MXfold2 [159]. These models modify the thermodynamic free energy parameters with data training through deep learning for the RNA triplet sequences, free energies, and their secondary structures, making them robust and highly accurate models, overcoming the limitations of the above models with minimum risk of overfitting the data [159].

9.1.2 Advanced models

Unlike conventional computational models, deep learning models are making a breakthrough in predicting the secondary structure of mRNA. They predict the secondary structure based on the binary classification of RNA sequences instead of making false assumptions about the structure, as the previous models do. Models that solve the problems of binary classification across RNA through deep learning approaches are IPknot [160], E2Efold [161], Ufold [162], and SPOT-RNA [163]. The first three train data sets use inter-programming and integer programming to predict the RNA base pairs. In contrast, SPOT-RNA determines the triplet RNA sequences, including other parameters, to avoid overfitting of data [163]. However, deep learning models require data training for many data sets, as they are designed by simply splitting the test and training datasets [164].

Among all the above models, the best fit to forecast the secondary structure is MXfold2 [159]. It integrates deep learning approaches and thermodynamics-based techniques, eventually becoming an advanced nearest neighbor model. It is based on the Zuker algorithm to determine the free energy value parameters, which allow high accuracy in evaluating stable secondary structure energies and free energies utilizing Turner's parameters.

The latest revolutionary deep learning model to break the barriers in neoantigen vaccine design is next-level DeepFoldRNA [165], which predicts the tertiary structure of RNA on the atomic level through geometric configurations. It overcomes the limitations of old models, which cannot predict the structure from sequences and require comparative simulations based on trained datasets for RNA prediction. It is based on self-attention neural networks coupled with gradient-based structure folding simulations to construct 3D structures from sequence alone. It observes and locates the restraints in geometric structures to process the sequences to build the RNA structure with Fletcher-Goldfarb-Shanno minimization computational analysis (L-BFGS) [166]. The working of DeepFoldRNA encompasses various steps: input feature generation, spatial geometric restraint prediction based on neural network architecture, and, lastly, L-BFGS simulations. The model repetitively searches the multiple sequences of RNA in databases through rMSA. Self-attention neural linkages create the inter-residue torsion angles and base pairwise distances. The neural network architecture comprises two transformers with multiple sequence alignment (MSA), encoded sequences on their surface, and positional embeddings of base pairs. The resultant geometric constraints are transformed into composites with the help of binned probability predictions and antilogs to direct the folding simulations in advance [165, 166].

Two types of datasets are evaluated through DeepFoldRNA: overly complex, non-redundant, and foldable 105 sets of structures of sequences of 70–250 nucleotides derived from 32 Rfam families. The other was a dataset of 17 non-redundant monomeric RNA structures obtained from a community-wide RNA puzzles experiment [165]. Researchers made 17 RNA puzzle experiments, out of which DeepFoldRNA made a breakthrough by obtaining the highest accuracy rate in 15 puzzle experiments. It achieved an average 2.69 RMSD score and 0.743 TM score in these experiments by a large margin compared to other models. The average errors were also negligible [165]. Although this model was launched two years ago, the updated version 2.0 is under development by experts to overcome the limitations [165, 166].

9.2 Optimization of vaccine delivery systems

To improve the efficiency of delivery and endosomal escape mechanisms, DL models can replicate interactions between LNPs and cellular membranes.

9.2.1 LNP delivery and AI-optimized nanoparticle design

Computational models mimic the behavior of LNP in biological systems to adjust their composition for effective distribution. They make the generative design of a lipid particle-based drug delivery system. iDRO-algorithms [167], Linear Design [168], UTR-LM, MXfold2 [169], and CNNs are the deep learning models applied to optimize the RNA sequences and LNP encapsulation efficiency with minimum risk of tangling of the RNA 2D structure. The first DL algorithm, Linear Design [168], is based on dynamic programming. Zhang et al. proposed the gene sequence DL model to optimize the mRNA gene sequence for the COVID-19 vaccine for the first time. This tool also improved mRNA half-life and protein expression by optimizing mRNA stability and codon use. It increased protein expression in mice by 128 times, along with mRNA half-life [168].

Integrated DL-based optimization algorithm (iDRO) [167] employs RNA-Bart and bidirectional long short-term memory networks (BiLSTMs), the two most

advanced types of neural networks, to optimize the RNA sequences, which exhibit about 1.2 times more translational efficiency than α -globins. RNA-Bart, in this algorithm, determines the mRNA sequences from validated design datasets through transformer-based learning. While BiLSTMs analyze the data sequences to predict mRNA sequences with long-range dependencies. MXfold2 also plays a vital role in improving mRNA stability and LNP formulation by predicting mRNA secondary structures [169].

CNN tools effectively evaluate mRNA sequences to identify mRNA secondary structures, motifs, knots, abundance, and other vital features directly affecting mRNA's translation efficiency and stability [170].

Wang et al. proposed a 5' UTR language model to identify the UTRs present at the 5' site in mRNA sequences for the prediction of mRNA secondary structures, mean ribosome loading (MRL), minimum free energy, protein expression level, and translation efficiency. Scientists created a dataset of 211 new 5' UTRs that can be decoded to achieve higher protein expression levels. The best-designed 5' UTR sequence obtained a 32.5% increase in protein expression level [171].

LNP simulations based on artificial intelligence frequently overlook intricate biological interactions that impact the immunity and viability of mRNA [172]. According to Mitchell et al., experimental validation is required to ensure that AI-designed LNPs function as anticipated [173]. Large datasets are necessary for deep learning models employed in LNP creation. However, there is currently a slight variation in the lipid formulations that are accessible [174].

9.3 Modulation of TME

Tumor microenvironment cell functions and their phenotypes are highly dependent on the cell interactions and precise cell allocations in spatial dimensions with the surrounding cells. In order to address the obstacles imposed by TME, artificial intelligence-mediated algorithms can provide suitable combination therapies that synergize with mRNA vaccines [175]. ML and DL tools are applied to modulate the tumor microenvironment in extremely malignant cancers. AI models are capable of estimating TME cell spatial distribution, cell classification, cell detection, identification, localization, quantification, gene expression variation, and disease-associated biomarkers [175].

9.3.1 Conventional models

Single and fusion ML and DL models for multidimensional characterization of TME are applied for cells' spatial characterization. A CNN developed by Zhu et al. implies the combination of spatial, multimodal, and boundary TME data to evaluate the brain tumor interrelationships and location with nearby tissues on MRI. Moreover, the model was validated to generate accurate results with enhanced efficiency [176].

CIBERSORTx, an extension of CIBERSORT, is one of the ML tools that examine the makeup of immune cells in tumors and helps create TME-modulating tactics in the mapping of TME through siRNA sequences in addition to mRNA vaccines by identifying the dominance of resistant M2 macrophages in mRNA vaccine therapy and also proved to be validated in melanoma trials [177]. However, the bulk RNA sequencing data used by CIBERSORT and related techniques may not have a single-cell resolution, which could result in inaccurate cell-type deconvolution and

incorrect immune cell population classification [177]. Despite the extended version of CIBERSORT to address the above limitations, the updated ML tool still has its limitations during TME analysis, which are reliance on predefined sequence RNA data, ignoring nonlinear genome interactions, failure to predict the spatial arrangement of tumor and immune cells, and inability to process the large omics datasets above 1,00,000+ cells.

9.3.2 Advanced models

The three types of deep learning models are working to overcome the limitations of the ML tools. Deep TME implies a CNN that predicts the tumor components' spatial dimensions. A DL tool based on MultiOmyx analysis [178] classifies and segments the TME images and calculates TME cell phenotypes, densities of cell phenotypes, biomarker information, and the morphological nature of TME cells. Additionally, the complex cell interaction in TME is determined by cell phenotype clustering patterns predicted by this model's advanced spatial analysis feature [178]. The fusion DL model, TransSea [179], containing CNNs with Transformers learning, can obtain 90.84% and 86.32% scores from BraTS2021 and BraTS2020.

Spatial transcriptomics data ensures in-depth knowledge and analysis of tumor and TME cells; however, complex use and high cost limit the usage of such data in clinical cases. A fusion deep learning model was proposed by Gao et al. to overcome the limitations of transcriptomics data usage. This model combines the power of GAN (IGI-DL), known as Generative Adversarial Network, to predict and analyze the transcriptomics data by processing structural features and image pixel intensities of hematoxylin eosinophil-stained images to ultimately predict the tumor heterogeneity and cancer prognosis. This model benchmarks against DeepSpace and HisToGene by showing the 179 predictive target genes and optimal performance in validated and test datasets. It can remarkably characterize the TME [180].

9.4 Biomarker discovery

The need for novel biomarkers and existing biomarkers for mRNA vaccine design is critical in the advanced stages of cancer. The transcriptome, genomic, and multi-omics data analysis results in the precision of cancer diagnosis and early-stage prevention [181]. AI-driven analysis of clinical data can discover potential new biomarkers for the assessment of therapeutic responses, ultimately enhancing the evaluation of vaccine safety and efficiency. Several types of biomarkers, such as genetic, imaging, and liquid biomarkers, can be discovered and predicted using machine learning and deep learning algorithms. Cerebro [182], one of the AI tools used to determine somatic mutation, is operated by rainforest algorithms with a high positive prediction value of 98% to estimate the level of circulating tumor DNA in the blood as a potential biomarker. The promising mRNA-based biomarker was established using a random survival forest algorithm and Cox proportional hazards regression to treat cutaneous melanoma [182].

DeepMirTar uses deep learning to discover miRNA-based biomarkers that affect tumor growth and vaccination response [183]. The miRNA prediction ultimately determines the tumor antigens. It combines long-term and short-term memory networks with CNNs to estimate potential miRNAs that modulate neoantigen immune-eliciting pathways. It is trained on cancer genome data with enhanced performance compared with other models [183].

9.5 Personalized mRNA-based cancer vaccines

Machine learning and deep learning algorithms can efficiently design promising personalized mRNA-based cancer vaccines based on varying tumor genomic data of individual patients. Both models can train on data and guide *in silico* tools to predict accurate results. ML and DL models such as NetMHCpan [184], MHCflurry [185], and DeepNeo [186] have integrated various features and algorithms to determine the neoantigens according to real-time patient data. However, vaccine customization requires in-depth analysis of all kinds of input data.

Heterogeneous datasets are incorporated *via* platforms such as *Deep Genomics* (<https://www.deepgenomics.com/>) to customize vaccine designs according to tumor profiles unique to each patient [172]. The main drawback of Deep Genomics and related platforms is their poor interpretability; deep learning models function as “black boxes,” making it challenging to comprehend the decision-making procedures underlying tailored vaccine recommendations [172].

9.6 Anticipation of neoantigens to cater to tumor heterogeneity

It is a fundamentally complex problem to locate the neoantigens that can elicit an immune response due to the presence of several factors, such as tumor heterogeneity, complexities in neoantigen-MHC-TCR interactions on the structural level, dynamic tumor evolution, scalability and practicality of personalized vaccines, and data sparsity through transfer learning. The conventional neoantigen identification algorithms consider all other features besides MHC-peptide binding affinity, such as mutation calling, MHC/HLA typing, TCR-peptide-MHC binding estimation, and forecasting of immunogenicity of promising neoantigens. They are based on artificial intelligence, machine learning, or neural networks [187].

Contrarily, deep learning-based models can potentially comprehend tumor genomic data to forecast neoantigens, aiding in overcoming tumor heterogeneity by identifying crucial biomolecular targets across various cancerous domains with enhanced precision and an accurate degree of neoantigen prediction. The high self-programming models can evaluate large multi-omics datasets such as RNA sequences, HLA typing, and whole exome sequencing (WES) to generate a robust immune response. These models identify and locate the potential neoantigens that are derived from gene fusions, insertions or deletions, alternative splicing events, and single-nucleotide variants (SNVs) by employing the convolutional neural networks (CNNs) and graph neural networks (GNNs) to broadly predict the neoepitopes that are less common across the tumor clones and subclones. Moreover, DL tools allow lower chances of human errors with modern computational technology's accelerated and dynamic approach toward results with fewer material and labor costs.

9.6.1 Neoantigen prediction from somatic mutations

The typical somatic mutations comprise single-nucleotide variants (SNVs), gene fusions [188], insertions or deletions (indels) [189], splice variants [190], intron retentions [191], non-coding regions [192], exon-exon junctions [193], and human endogenous retroviruses.

The most common source of neoantigen prediction is SNV. The machine learning tools for forecasting neoantigens are LumosVar [194], DeepVariant, GATK 4.0 [195], Cerebro [196], and SpeedSeq [197]. LumosVar, also known as the Bayesian

tumor-only somatic variant caller, predicts the variant by analyzing the mutational rate between somatic and germline tumor samples. However, it is highly dependent on sequencing depth and sample purity. However, the predicted neoantigens are not more immunogenic than those predicted from non-SNV sources, as the latter source involves rapid mutations in protein sources. The paired tumor samples from humans, such as standard adjacent and tumor samples, cannot always be analyzed [198]. Wood et al. implied a random forest approach to evaluate the large dataset to generate a reliable confidence value for each mutation. The model provides accurate results with a predictive value of 98% compared to other models due to its automation, which uses accurate word training data from representative sources. The only limitation of NGS datasets [196].

Google employed deep neural networks in DeepVariant [197] to predict the genetic mutations of sequenced input through a tumor image classification model. It benchmarks against SpeedSeq and GATK 4.0, a standard computational model for predicting neoantigens due to high F-score values such as 99.40% from indels and 99.96% from SNVs, as reported by the FDA [195]. DeepVariant constructs side-by-side images of standard and tumor samples at each mutation site. The convolutional neural network recognizes image models to detect the biological variations in sequences. However, data analysis and neoantigen prediction are very time-consuming. Its updated version, DeepVariant-on-Spark [199], takes relatively less time due to its upgraded feature, which optimizes and distributes the input data in less time. NeuSomatic [200] is another DL tool composed of nine deep convolutional neural network layers in the network architecture. Other features, such as data alignment for model training to directly extract information from raw sequencing data, are present in this tool, unlike in DeepVariant. It takes feedback from other detection methods and integrates results into its input compared to its version, NeuSomatic-S. Furthermore, operating on 156 CPU core-hours for data training, more than 1000 CPU core-hours, supporting GPUs, and inferring output results in PyTorch are the additional features that make it outperform in determining neoantigen in smaller allelic tumor samples. However, NeuSomatic relies on a higher processing time than DeepVariant [200].

The most accurate DL model is DeepNeo, which applies multiple models of deep learning to integrate several features, such as mutations in allelic frequencies, gene expression, and structural interactions. It operates on transfer learning and attention networks comprising 3D CNNs and GNNs to train peptide and TCR sequences. The optimistic prediction value is 94% in comparison with other models. The clinical trials demonstrated a 44% success rate in melanoma patients administered with vaccines designed on this model [201]. It is believed that neoantigen candidates originating from non-SNVs are potentially more immunogenic and can influence cancer immunotherapy effectively. Still, much work is required to predict the fusion gene-derived neoantigens using artificial intelligence [202].

9.6.2 Neoantigen prediction from HLA typing

MHC alleles vary individually in the population and, thus, can affect the peptide-MHC binding affinity. MHC class I and class II have different binding grooves for peptides on their binding site, which HLA genotyping affects. MHC class I has a closed binding groove at both ends, which targets unvarying sequences at the backbone of a peptide with 8–11 amino acids to assist the neoantigen in binding with TCR from the MHC I complex to TCR through its variable sequences [203]. Conversely,

an open binding groove is present at MHC II, which facilitates the binding of MHC II with large peptides having 12–20 amino acid sequences. The open binding groove causes difficulty in accurately estimating the binding affinity of the peptide-MHC II complex [198]. Predicting the patient alleles through HLA typing is crucial before estimating peptide-MHC binding affinity. HLA typing directly generates different MHC haplotypes with varying degrees of affinity for neoantigens. PCR-SBT is a standard tool for estimating HLA genotyping but cannot analyze heterozygous or polymorphic HLA alleles [204].

The traditional ML algorithms, such as seq2HLA [205], OptiType [206], PolySolver [207], and PHLAT [208], ensure an economical approach and provide the highest accuracy rate compared with the gold standard using RNA or DNA sequences. OptiType is an extremely sensitive tool that selectively predicts HLA type I with WES or RNA sequence data. Seq 2HLA predicts HLA type I and II using RNA sequences [205]. PHLAT also predicts HLA I and II genotypes from RNA sequences or WES data [208]. PolySolver is a widely used HLA I prediction tool using WES data, but it is limited [206].

The deep learning tool is HLAScan [209], which processes raw sequencing data with CNNs and predicts HLA class I alleles with a 99% positive prediction rate. ATHLATES bidirectionally employ long short-term memory networks (LSTMs) to predict HLA I and II. The most accurate DL tool combines CNNs and GNNs to process the polymorphic HLA I and II genotypes. It makes a graph for each allele, taking edges as linkage disequilibrium and nodes encoding exons using whole genome sequencing (WGS) data. It quickly identifies HLA alleles in the population dataset and predicts the neoantigen peptide binding region. It improves the estimation of neoantigen prediction with an 18% success rate in designed vaccines against melanoma [209].

9.6.3 Neoantigen prediction from peptide-MHC I & II binding affinity

It is an essential feature to extract neoantigens by estimating the binding affinity of neoantigens with MHC I and II. The previous tools were solely based on matrix-based approaches and linear regression models. The current approaches to predicting peptide-MHC binding affinity apply ML and DL models, which evaluate the non-linear relationship between MHC molecules and neoantigen sequences.

In order to predict how well neoantigens bind to MHC molecules, which is a crucial step in initiating an immune response, tools such as NetMHCpan 4.0 [210] employ allele-specific artificial neural networks (ANNs) and machine learning algorithms on peptide-HLA binding data. It is trained on approximately 180,000 mass spectrometry HLA-peptide binding affinity quantitative data. One of the most significant drawbacks of this tool and similar methods is reduced universality to uncommon or new HLA alleles due to excessive dependence on training datasets, which tend to lean toward well-characterized and already reported alleles [211].

To find putative cancer neoantigens, EpiToolkit [212] is a neoantigen prediction pipeline that analyzes whole genome sequencing (WGS), whole exome sequencing (WES), and RNA-seq data, i.e., single-nucleotide variants (SNVs) and indels. It utilizes NetChop [213] (an artificial neural network) for proteasomal cleavage analysis and NetMHC [214] (an artificial neural network (ANN)) for peptide-MHC binding affinity prediction. It facilitates the identification of immune-stimulating structures by investigating HLA types and peptide sequences [212]. The pipeline supports immunological research by concentrating on MHC class I neoantigen predictions [212].

EpiToolkit's accuracy in predicting odd or unique neoantigens could be limited by its reliance on pre-established HLA binding affinity models [212]. Furthermore, it does not incorporate posttranslational modification (PTM) studies, which can influence the projected neoantigens' capacity to elicit an immune response [212].

In order to predict MHC class I neoantigens, pVAC-Seq analyzes WES and RNA-seq data [215]. It assesses sequence coverage and predicts the peptide-MHC binding affinity *via* NetMHC v3.4 [216]. The pipeline recruits Cufflinks [217] for gene expression profiling and bam-readcount [218] for allele variation frequency exploration. This all-encompassing method improves the discovery of neoantigens for tailored cancer immunotherapy. Since peptide processing efficiency and posttranslational modifications are essential for precise neoantigen identification, pVAC-Seq mainly depends on projected MHC binding affinity and variant allele frequency [215]. Sequencing discrepancies and inconsistencies in variant calling might also impact its performance [215]. FRED2 [219] predicts both MHC class I and II neoantigens by employing WES and RNA-seq data and evaluates the peptide-MHC binding affinity through the integration of several methods, such as NetMHC [216] and NetMHCpan. Protease cleavage prediction, mutation coverage, frequency of mutant alleles, gene expression, and assessment of TAP transport efficiency are further aspects of this approach. This multistep system incorporates a variety of pathogenicity parameters to improve neoantigen detection [219]. Although FRED2 incorporates several forecasting approaches, it is still constrained by the completeness and quality of the available training data, which could result in errors in the ranking of neoantigens. Furthermore, it fails to consider the intricacies of antigen presentation and processing, which may lead to false-positive results [219].

With an emphasis on SNV-based neoantigen prediction, CloudNeo [220] examines variant call format (VCF) files for mutations and BAM files for HLA typing and assesses the peptide-MHC class I binding affinity using NetMHCpan. The process detects plausible neoantigens by combining mutation and HLA type data [220]. This simplified method predicts immunogenic peptides to assist in tailored cancer immunotherapy [220]. Prediction accuracy may be decreased by CloudNeo's inadequate incorporation of peptide structural and biological properties, which is centered on SNV-based neoantigen prediction. Furthermore, it uses NetMHCpan to predict binding affinities, which might not adequately account for the complexity of antigen presentation across a range of HLA [220].

Tlminer [221] calculates the peptide-MHC binding affinity leveraging RNA-seq data, particularly SNVs. RNA-seq readings are fed into the Kallisto tool [222], which measures gene expression in the first phase; then, taking peptide sequences and HLA type as input, the NetMHCpan tool determines the peptide-MHC binding probability. To better comprehend the interactions, the model concentrates on MHCI binding affinities. Tlminer does not thoroughly evaluate elements essential for immunogenicity, such as peptide processing, transport, and posttranslational modifications, and instead concentrates on MHC I binding prediction. Its dependence on RNA-seq data may also generate biases because of sequencing errors and variability in expression [221].

The INTEGRATENeo [223] pipeline uses RNA-seq and WGS data to emphasize gene fusions. The NetMHC4 tool employs peptide sequences and HLA type as input to predict the peptide-MHC binding affinity. In order to shed light on how peptides and MHC molecules interact for both classes of MHC, the model forecasts both MHCI and MHCII binding affinities [223].

Gene fusion-derived neoantigens are the primary focus of INTEGRATENeo, which may miss important immunogenic targets due to its weak ability to predict neoantigens from SNVs and indels [223]. Furthermore, it uses NetMHC4 to forecast binding affinities, which might not adequately account for the intricacies of antigen presentation and processing [223].

SNVs, indels, and splicing variations are the primary objective of the NeoantigenR [224] process, which employs DNA and RNA-seq data. The NetMHC tool analyzes peptide sequences and HLA type as input to predict the peptide-MHC binding affinity, and by examining peptide-MHC interactions, the model forecasts explicit MHCI binding affinities to identify putative neoantigens. NeoantigenR [224] does not include structural modeling of peptide-MHC interactions, which can influence immunogenicity estimates, instead concentrating on SNVs, indels, and splicing changes. Furthermore, because rare HLA alleles are not well covered in training datasets, their reliance on NetMHC for binding affinity prediction may induce biases [224].

SNVs and indels are the key objectives of the MuPeXI [225] process, which employs WES data, and by considering peptide sequences and HLA type as input files, the pipeline leverages the NetMHCpan 3.0 tool to predict peptide-MHC binding affinity. Along with taking into account the frequency of mutant alleles, the standard exact match penalty, and the number of mismatches between mutant and normal peptides, it also incorporates gene expression data. In order to determine possible neoantigens and their immunogenicity, the model focuses on MHCI binding affinities. Although alternative splicing events and posttranslational modifications can affect neoantigen immunogenicity, MuPeXI concentrates on SNV and indel-derived neoantigens. Furthermore, biases could arise from the dependence on NetMHCpan for peptide-MHC binding affinity predictions, especially for rare HLA alleles [225].

The Vaxrank [226] pipeline targets SNVs and indels, deploying RNA-seq data. Peptide sequences and HLA type are inputs, and the NetMHCpan tool is recruited to predict the peptide-MHC binding affinity. Gene expression analysis is included in the list of possible neoantigens to further scrutinize the list of possible neoantigens. The model primarily looks for interesting vaccination candidates based on MHCI binding affinities. Vaxrank ranks neoantigens according to their MHC binding capacity and RNA expression, but it ignores elements vital to immunogenicity, such as peptide processing, stability, and posttranslational modifications. Furthermore, biases may be introduced by its extensive reliance on NetMHCpan for binding predictions, especially for uncommon HLA alleles [226].

This pipeline focuses on indels and employs RNA-seq data [227], where both the NetMHC and NetMHCpan utilize peptide sequences and HLA type as input to predict the peptide-MHC binding affinity. Using the Ensembl Variant Effect Predictor tool [228], which examines recognized indels, the pipeline additionally considers the variant allele frequency and the fold difference between wild-type and mutant alleles. The model evaluates possible neoantigen candidates by focusing on MHCI binding affinities [227].

Peptide processing and transport systems, essential for antigen presentation, are not entirely incorporated by ScanNeo, which concentrates on indel-derived neoantigens [227]. Furthermore, biases may be introduced by its reliance on NetMHC and NetMHCpan for binding predictions, especially for uncommon HLA alleles with little training data [227].

This pipeline, which emphasizes SNVs and indels, benefits from somatic variant calls. The NetMHCpan tool combines peptide sequences and HLA type as input

feed to predict the peptide-MHC binding affinity. By determining the amplitude of the ratio of the relative probabilities of binding for the wild-type and mutant epitopes to MHC I molecules and a metric of resemblance to pathogenic peptides, it also evaluates the potential for neoantigen recognition. In order to forecast neoantigen recognition, the model relies on MHCI binding affinities. While post-translational modifications and non-coding RNA-derived peptide molecules might affect immunogenicity, NeoPredPipe mainly concentrates on SNV and indel-derived neoantigens. Furthermore, because rare HLA alleles are not well represented in the training datasets, their reliance on NetMHCpan for binding predictions may create biases [229]. SNVs and indels are the primary motivations of the Neoepiscope [230] pipeline, which employs DNA-seq data. Both the MHCflurry neural network [185] and the NetMHCpan tool recruit peptide sequences and HLA type as input to estimate the peptide-MHC binding affinity. In order to determine possible neoantigens and their immunogenicity, the model forecasts MHCI and MHCII binding affinities [230]. Although Neoepiscope incorporates multivariant phasing for neoantigen prediction, it does not entirely consider the impact of peptide breakdown or posttranslational changes, which can alter immunogenicity [230]. Furthermore, because of the restricted diversity of HLA alleles in training datasets, its reliance on NetMHCpan and MHCflurry for binding predictions may induce biases [230].

With an emphasis on SNVs, indels, and gene fusions, the pVACtools pipeline renders utilization of somatic variant calls, and by using peptide sequences and HLA type as input, it ranks the peptide-MHC binding affinity using techniques like MHCflurry, NetMHC, NetMHCpan, and NetMHCIIpan [231]. Using the Ensemble Variant Effect Predictor tool, the pipeline additionally ranks the protein variant allele percentage, mutant allele expression, and fold change between mutant and wild-type alleles. In order to evaluate neoantigen potential, both MHCI and MHCII binding affinities must be considered. Although it offers a complete toolkit to forecast neoantigens, pVACtools is constrained by its dependence on anticipated MHC binding affinity, which does not necessarily indicate true immunogenicity. Furthermore, the pipeline does not completely incorporate elements that can impact neoantigen appearance and recognition, such as alternative splicing or posttranslational modifications.

The neoANT-HILL [232] method targets SNVs and indels, deploying WGS, WES, or RNA-seq data, and utilizes the Kallisto tool to process RNA-seq readings and perform gene expression analysis. With peptide sequences and HLA type as input, peptide-MHC binding affinity is predicted using MHCflurry, NetMHC, NetMHCpan, and NetMHCIIpan. The model predicts probable neoantigens by concentrating on MHCI binding affinities [232]. NeoANT-HILL aims to find putative neoantigens but does not fully integrate posttranslational modifications and antigen-processing kinetics, essential for precise immunogenicity predictions. Furthermore, because different algorithms provide different predictions, their dependence on several binding prediction tools, like NetMHCpan, could result in discrepancies [232].

The NeoFuse platform emphasizes gene fusions utilizing RNA-seq data and processes RNA-seq readings along with gene expression analysis using the STAR [233] and featureCounts [233] bioinformatic programs. The MHCflurry neural network uses peptide sequences and HLA type as input to estimate the peptide-MHC binding affinity. In order to find possible neoantigens, the model focuses on MHCI binding affinities.

The ASNEO [234] pipeline prioritizes alternative splicing and employs RNA-seq data. With peptide sequences and HLA type as input, the NetMHCpan-4.0 tool

estimates the peptide-MHC binding affinity. Along with a combined score of binding affinity, proteasomal C-terminal cleavage, and TAP transport efficiency using NetCTLpan [235], the pipeline also considers the number of mismatches between mutant and standard peptides. It combines a T-cell recognition score with a hydrophobicity value based on peptide hydrophobicity. MHCI binding affinities are the primary focus of the model [234].

ASNEO concentrates on neoantigens formed from alternative splicing but does not fully incorporate other mutation types like SNVs and indels, which could lead to omitting important immunogenic targets. Furthermore, biases may be introduced by the dependence on NetMHCpan for binding affinity predictions, especially for rare HLA alleles in training datasets [234].

SNVs and indels are the primary concerns of the VENUS [236] pipeline, incorporating WES and RNA sequencing data. It examines the number of transcripts containing mutations and the allele frequency of those mutations. The consensus technique from IEDB2.17 software [237], which evaluates MHCI binding affinities to predict neoantigen potential, is used to predict peptide-MHC binding affinity.

Although VENUS incorporates transcriptome data for enhanced neoantigen prediction, it fails to account for peptide breakdown or posttranslational changes, which can affect immunogenicity [238]. Furthermore, biases may be introduced by the dependence on consensus approaches for predicting MHC binding affinity, especially for uncommon or poorly understood HLA alleles [236].

The NeoSplice [239] pipeline focuses on alternative splicing and leverages RNA-seq data. With peptide sequences and HLA type as input, it uses NetMHCpan-4.0 and NetMHCIIpan-3.2 to predict peptide-MHC binding affinity. In order to evaluate possible neoantigens, the model forecasts both MHCI and MHCII binding affinities [239]. NeoSplice concentrates on neoantigens generated through alternative splicing, but it does not entirely incorporate SNVs and indels, therefore missing important immunogenic targets. Furthermore, biases may be introduced by the dependence on NetMHCpan and NetMHCIIpan for binding capacity estimates, especially for uncommon HLA alleles with little training data [239].

Neoantigens are ranked according to their immunogenic potential by algorithms like DeepImmuno [240], which aid in prioritizing vaccine development candidates. This model utilizes graph neural networks (GNNs) on neoantigen-MHC structural complexes to generate the structural immunogenicity by modeling 3D neoantigen peptide-MHC interactions. This DL tool benchmarks against other models because it integrates the T-cell receptor (TCR) recognition pattern and model and predicts the 3D dynamics of peptide-MHC.

The graph neural networks and attention architecture prioritize peptide-HLA binding and focus on high-affinity binders in DeepImmuno. It creates the graph of the peptide-MHC complex, where the edges represent complex physicochemical and spatial interactions. In contrast, nodes encode for amino acid residues, allowing TCR docking alignment for immunogenicity, flexibility in conformational structures of neoantigen binding grooves, and allosteric site flexibility of PTMs. It is validated and trained on GTEx and TCGA datasets, where it predicts the binding energy with a 0.2 Å RMSD accuracy value, obtaining 93% prediction of potential neoantigens compared to other models with a false-positive rate of 7%. It also allows for incorporating other model features such as cryo-EM, single-cell RNA sequence data, and mass spectrometry, thus creating multimodal training for datasets. DeepImmuno model-based vaccines have shown a success rate in 40% of patients undergoing melanoma trials compared to 18% response *via* NetMHC-based vaccines. In the

ELISPOT assay, the predicted neoantigens demonstrated 2.5 times T-cell activation. It also rapidly identifies the MHC II neoantigens to overcome the stromal barriers in pancreatic cancer.

According to Marzella et al., peptide-MHC predictions are still limited by a lack of experimentally validated empirical binding data, even with advancements in geometric deep learning. DeepImmuno, along with other deep learning-based algorithms such as pVAC, tends to overfit existing datasets, which reduces their accuracy in predicting unknown neoantigens [241]. According to Kim et al., experimental confirmation or structural data is essential, as ImmunoDeep still has trouble with false-positive predictions [241]. These limitations can be tackled by combining GNNs of DeepImmuno with pVACtools for maximum coverage and analysis of neoantigen-MHC II complexes. Furthermore, the neoANT-HILL integration allows the adoption of scRNA sequences to predict clonal neoantigens.

9.6.4 Neoantigen prediction from TCR-pMHC binding affinity

Once the potential neoantigen is explored, validating through *in vitro* or *in silico* tools is essential to predict whether the candidate neoantigens can stimulate T-cells to eradicate cancerous cells. The available machine learning and deep learning tools for peptide-TCR binding affinity prediction are NetTCR 2.0 [186], pMTnet [242], and ERGO [243]. However, several tools are being evaluated due to inadequate information being available.

NetTCR 2.0 uses CNN to predict peptide-TCR binding affinity by utilizing the paired data of TCR α and β . The input data of peptide CDR3 α and β units are added to CNNs. The BLOSUM 50 matrix encodes amino acids. Max pooling and one-dimensional convolutional layers encode the amino acid, and the sigmoid function generates output results in dense layers. NetTCR provides accurate results with a 79% positive prediction value and a 2% false rate compared to other models. Despite training both sets of TCR α and β , the main limitation of this model is the analysis of specific types of 9mer peptides and HLA-A*02:01 [186].

The transfer learning-based tool to predict TCR affinity with the pMHC complex is pMTnet [242], which uses the recognized antigens originating from the MHC sequence, T lymphocyte and cancerous cell complex binding, and TCR sequence. It is applied in deep learning models for their training. Its accuracy was reported to be up to 0.827 when an independent dataset was used. Due to biased results, pMTnet is highly accurate compared to other models such as TCRGP, NetTCR, and TCRGP [242].

Springer developed deep learning methods such as ERGO [243] to evaluate the interaction between peptide-TCR complexes. It used a short-term memory deep learning algorithm to encode whole peptide sequences and CDR3 for neural network training. Two output conditions are set: 0 represents no binding, and 1 represents peptide binding with TCR. Its performance and accuracy are similar to the other standard methods. MHC data is inapplicable in this model, which causes its limited application in peptide-TCR binding prediction [243].

9.6.5 Candidate neoantigen immunogenicity prediction

The in-depth mechanism of an epitope eliciting an immune response by T-cells is still unclear. However, researchers reported that the immunogenicity of predicted

neoantigens is dependent on several factors, including epitope sequence, the molecular size of the peptide, amino acid polarity, peptide entropy, the hydrophobic nature of the peptide and TCR, tumor abundance potential, and the potential contact of amino acid pair residues. Various features are integrated into AI tools to determine the accurate estimation of the immunogenic potential of candidate neoantigens.

Neopepsee [244] employs NGS data to identify the immunogenic potential through ML classifiers such as random forest, SVMs, Gaussian Naïve Bayes, and locally weighted naïve Bayes. The nine immunogenic features are added, ranging from hydrophobicity, charge, polarity, immunogenicity score, combined scores, IC₅₀ value of pMHC binding interaction, percentage rank of pMHC binding affinity, similarity index to known neoantigens, differential agretopicity index (DAI), and amino acid pairwise contact potential (AAPP). Using an independent dataset, Neopepsee demonstrated improved results in patients with chronic lymphocytic leukemia (CLL) and melanoma with an F-score of 0.48–0.56 [244]. pTuneos [245] is a 20 times faster *in silico* approach than Neopepsee and comparable in performance to pVAC-Seq [246] and MuPeXI [225]. It prioritizes neoantigens and has proved effective against many datasets, such as tumor checkpoint inhibitor treatments, tumor-infiltrating lymphocyte (TIL) datasets and the cancer genome atlas (TCGA) datasets. However, a requirement of a large number of trained and tested datasets and relatively fewer immunogenic features influences its performance and accuracy [22].

Ineo-Epp [238] works on a supervised machine learning algorithm known as random forest to determine the neopeptides' immunogenicity. It combines and integrates up to 24 immunogenic features of candidate neoantigens covering peptide structure, amino acids' physicochemical characteristics, % rank of peptide-MHC binding affinity, agretopicity index, and peptide entropy, along with fivefold cross-validation to accurately determine the true neoantigens. However, its training on small datasets limits its reliability.

TruNeo [247], a deep learning model with a scoring scheme, best estimates the prediction of high-confidence neo-peptides with true immunogenicity. It forecasts neoantigen immunogenicity based on six features: HLA loss of heterozygosity, tumor heterogeneity, antigen transporter efficiency, MHC binding affinity, expression abundance, pMHC binding affinity, and clonality. TruNeo outperforms MHCflurry by 2.5 times in lung cancer patients, showing up to 50% more positive predictive results than MHCflurry. However, data validation using one actual patient was employed to compare these two models.

DeepImmuno-CNN [248] applies CNNs based on PCA encoding strategy and AAindexI usages on data to determine true neoantigens, their 29 immunogenic features, and residues crucial for recognizing antigens. Only this deep learning model can predict many immunogenicity features out of 35, even more than the DeepHLApan and IEDB databases. However, the shortage of available matched TCR sequence data and prediction through only peptide data influence its accuracy and performance.

Although the ML and DL fields are progressing at a fast pace to overcome the challenges in designing neoantigen-based mRNA cancer vaccines, the lack of comparative studies, comprehensive studies using AI tools, and key limitations of deep learning models still need much work before utilizing the full potential of deep learning tools in combating cancer.

10. Conclusion

mRNA cancer vaccines annihilate the tumor cells through precise targeting and eliciting the immune response. Artificial intelligence's computational advancement witnesses mRNA vaccines' potential future in cancer immunotherapy. By harnessing machine learning and deep learning models in addressing the challenges of mRNA cancer vaccine design, they have garnered attention for treating cancer with enhanced safety, optimized efficacy, and personalized treatment options.

Author details

Saba Ismail and Khaled Barakat*
Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Canada

*Address all correspondence to: kbarakat@ualberta.ca

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Baxevanis CN, Perez SA, Papamichail M. Cancer immunotherapy. *Critical Reviews in Clinical Laboratory Sciences*. 2009;**46**(4):167-189
- [2] Restifo NP, Dudley ME, Rosenberg SA. Adoptive immunotherapy for cancer: Harnessing the T cell response. *Nature Reviews. Immunology*. 2012;**12**(4):269-281
- [3] Luo C, Han W. Harnessing the immune system to control cancer. *Chinese Science Bulletin*. 2016;**61**(26):2876-2882
- [4] Guo C, Manjili MH, Subjeck JR, Sarkar D, Fisher PB, Wang XY. Therapeutic cancer vaccines. In: *Advances in Cancer Research*. Elsevier; 2013. pp. 421-475. Available from: <https://linkinghub.elsevier.com/retrieve/pii/B9780124071902000071>
- [5] Coban C, Kobiyama K, Aoshi T, Takeshita F, Horii T, Akira S, et al. Novel strategies to improve DNA vaccine immunogenicity. *CGT*. 2011;**11**(6):479-484
- [6] Beck JD, Reidenbach D, Salomon N, Sahin U, Türeci Ö, Vormehr M, et al. mRNA therapeutics in cancer immunotherapy. *Molecular Cancer*. 2021;**20**(1):69
- [7] Boczkowski D, Nair SK, Snyder D, Gilboa E. Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. *The Journal of Experimental Medicine*. 1996;**184**(2):465-472
- [8] Fong L, Brockstedt D, Benike C, Wu L, Engleman EG. Dendritic cells injected via different routes induce immunity in cancer patients. *The Journal of Immunology*. 2001;**166**(6):4254-4259
- [9] Weide B, Pascolo S, Scheel B, Derhovannessian E, Pflugfelder A, Eigentler TK, et al. Direct injection of protamine-protected mRNA: Results of a phase 1/2 vaccination trial in metastatic melanoma patients. *Journal of Immunotherapy*. 2009;**32**(5):498-507
- [10] Martinon F, Krishnan S, Lenzen G, Magné R, Gomard E, Guillet J, et al. Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. *European Journal of Immunology*. 1993;**23**(7):1719-1722
- [11] Guan S, Rosenecker J. Nanotechnologies in delivery of mRNA therapeutics using nonviral vector-based delivery systems. *Gene Therapy*. 2017;**24**(3):133-143
- [12] Xue T, Stavropoulos E, Yang M, Ragno S, Vordermeier M, Chambers M, et al. RNA encoding the MPT83 antigen induces protective immune responses against mycobacterium tuberculosis infection. *Infection and Immunity*. 2004;**72**(11):6324-6329
- [13] Sabnis S, Kumarasinghe ES, Salerno T, Mihai C, Ketova T, Senn JJ, et al. A novel amino lipid series for mRNA delivery: Improved endosomal escape and sustained pharmacology and safety in non-human primates. *Molecular Therapy*. 2018;**26**(6):1509-1519
- [14] Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA vaccine against SARS-CoV-2—Preliminary report. *The New England Journal of Medicine*. 2020;**383**(20):1920-1931
- [15] Eisenächer K, Steinberg C, Reindl W, Krug A. The role of viral nucleic acid recognition in dendritic cells for innate

and adaptive antiviral immunity. *Immunobiology*. 2008;**212**(9-10):701-714

[16] Kreiter S, Diken M, Selmi A, Diekmann J, Attig S, Husemann Y, et al. FLT3 ligand enhances the cancer therapeutic potency of naked RNA vaccines. *Cancer Research*. 2011;**71**(19):6132-6142

[17] Wei J, Hui AM. The paradigm shift in treatment from Covid-19 to oncology with mRNA vaccines. *Cancer Treatment Reviews*. 2022;**107**:102405

[18] Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. *Nature*. 2015;**519**(7543):366-369

[19] Blass E, Ott PA. Advances in the development of personalized neoantigen-based therapeutic cancer vaccines. *Nature Reviews Clinical Oncology*. 2021;**18**(4):215-229

[20] Rajasagi M, Shukla SA, Fritsch EF, Keskin DB, DeLuca D, Carmona E, et al. Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia. *Blood, The Journal of the American Society of Hematology*. 2014;**124**(3):453-462

[21] Brown SD, Warren RL, Gibb EA, Martin SD, Spinelli JJ, Nelson BH, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Research*. 2014;**24**(5):743-750

[22] Kim S, Kim HS, Kim E, Lee MG, Shin EC, Paik S. Neopepsee: Accurate genome-level prediction of neoantigens by harnessing sequence and amino acid immunogenicity information. *Annals of Oncology*. 2018;**29**(4):1030-1036

[23] He Q, Gao H, Tan D, Zhang H, Wang J, zhi. mRNA cancer vaccines: Advances, trends and challenges. *Acta Pharmaceutica Sinica B*. 2022;**12**(7):2969-2989

[24] Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature*. 2012;**482**(7385):400-404

[25] Saha A, Kaul R, Murakami M, Robertson ES. Tumor viruses and cancer biology: Modulating signalling pathways for therapeutic intervention. *Cancer Biology & Therapy*. 2010;**10**(10):961-978

[26] Lee S, Yoon H, Hong SH, Kwon SP, Hong JJ, Kwak HW, et al. mRNA-HPV vaccine encoding E6 and E7 improves therapeutic potential for HPV-mediated cancers via subcutaneous immunization. *Journal of Medical Virology*. 2023;**95**(12):e29309

[27] Oğuz F, Atmaca H. mRNA as a therapeutics: Understanding mRNA vaccines. *Advanced Pharmaceutical Bulletin*. 2021;**12**(2):274

[28] Dong Y, Anderson DG. Opportunities and challenges in mRNA therapeutics. *Accounts of Chemical Research*. 2022;**55**(1):1-1

[29] Pardi N, Hogan MJ, Weissman D. Recent advances in mRNA vaccine technology. *Current Opinion in Immunology*. 2020;**65**:14-20

[30] Lundstrom K. RNA viruses as tools in gene therapy and vaccine development. *Genes*. 2019;**10**(3):189

[31] Self-replicating RNA viruses for RNA therapeutics. Available from: <https://www.mdpi.com/1420-3049/23/12/3310>

- [32] Ljungberg K, Liljeström P. Self-replicating alphavirus RNA vaccines. *Expert Review of Vaccines*; **14**(2):177-194. DOI: 10.1586/14760584.2015.965690
- [33] mRNA-based therapeutics: powerful and versatile tools to combat diseases. *Signal Transduction and Targeted Therapy*. Available from: <https://www.nature.com/articles/s41392-022-01007-w>
- [34] Yang W, Deng H, Zhu S, Lau J, Tian R, Wang S, et al. Size-transformable antigen-presenting cell-mimicking nanovesicles potentiate effective cancer immunotherapy. *Science Advances*. 2020;**6**(50):eabd1631
- [35] Sun Z, Jing C, Zhan H, Guo X, Suo N, Kong F, et al. Identification of tumor antigens and immune landscapes for bladder urothelial carcinoma mRNA vaccine. *Frontiers in Immunology*. 2023;**14**
- [36] Tang Y, Fan W, Chen G, Zhang M, Tang X, Wang H, et al. Recombinant cancer nanovaccine for targeting tumor-associated macrophage and remodeling tumor microenvironment. *Nano Today*. 2021;**40**:101244
- [37] Hydrogel-guided strategies to stimulate an effective immune response for vaccine-based cancer immunotherapy | *Science Advances*. DOI: 10.1126/sciadv.adc8738
- [38] Kim SC, Sekhon SS, Shin WR, Ahn G, Cho BK, Ahn JY, et al. Modifications of mRNA vaccine structural elements for improving mRNA stability and translation efficiency. *Molecular & Cellular Toxicology*. 2022;**18**(1):1-8
- [39] Schoenmaker L, Witzigmann D, Kulkarni JA, Verbeke R, Kersten G, Jiskoot W, et al. mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability. *International Journal of Pharmaceutics*. 2021;**601**:120586
- [40] A comprehensive review of mRNA vaccines. Available from: <https://www.mdpi.com/1422-0067/24/3/2700>
- [41] Perenkov AD, Sergeeva AD, Vedunova MV, Krysko DV. In vitro transcribed RNA-based platform vaccines: Past, present, and future. *Vaccine*. 2023;**11**(10):1600
- [42] Pardi N, Muramatsu H, Weissman D, Karikó K. In vitro transcription of long RNA containing modified nucleosides. In: Rabinovich PM, editor. *Synthetic Messenger RNA and Cell Metabolism Modulation*. Vol. 969. Totowa, NJ: Humana Press; 2013. pp. 29-42
- [43] Rosa SS, Prazeres DMF, Azevedo AM, Marques MPC. mRNA vaccines manufacturing: Challenges and bottlenecks. *Vaccine*. 2021;**39**(16):2190-2200
- [44] mRNA capping: Biological functions and applications | *Nucleic Acids Research*. Oxford Academic. Available from: <https://academic.oup.com/nar/article/44/16/7511/2460195>
- [45] Daffis S, Szretter KJ, Schriewer J, Li J, Youn S, Errett J, et al. 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. *Nature*. 2010;**468**(7322):452-456
- [46] A nuclear cap binding protein complex involved in pre-mRNA splicing: *Cell*. Available from: [https://www.cell.com/cell/abstract/0092-8674\(94\)90530-4](https://www.cell.com/cell/abstract/0092-8674(94)90530-4)
- [47] Li M, Wang Z, Xie C, Xia X. Advances in mRNA vaccines. In: Aranda F, Berraondo P, Galluzzi L, editors. *International Review of Cell and Molecular Biology*. Academic Press; 2022.

pp. 295-316. Available from: <https://www.sciencedirect.com/science/article/pii/S1937644822000569>

[48] To KKW, Cho WCS. An overview of rational design of mRNA-based therapeutics and vaccines. *Expert Opinion on Drug Discovery*. 2021;**16**(11):1307-1317

[49] Novel “anti-reverse” cap analogs with superior translational properties. Available from: <https://rnajournal.cshlp.org/content/9/9/1108.short>

[50] Tanguay RL, Gallie DR. Translational efficiency is regulated by the length of the 3' untranslated region. *Molecular and Cellular Biology*. 1996;**16**(1):146-156

[51] Leppek K, Das R, Barna M. Functional 5' UTR mRNA structures in eukaryotic translation regulation and how to find them. *Nature Reviews. Molecular Cell Biology*. 2018;**19**(3):158-174

[52] Overexpression of Urokinase receptor in mammalian cells following administration of the in vitro transcribed encoding mRNA. *Gene Therapy*. Available from: <https://www.nature.com/articles/3300930>

[53] Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells | *Blood*. American Society of Hematology

[54] Bossi L, Roth JR. The influence of codon context on genetic code translation. *Nature*. 1980;**286**(5769):123-127

[55] Liu Q. Comparative analysis of base biases around the stop codons in six eukaryotes. *Biosystems*. 2005;**81**(3):281-289

[56] Kudla G, Lipinski L, Caffin F, Helwak A, Zylicz M. High guanine and cytosine content increases mRNA levels in mammalian cells. *PLoS Biology*. 2006;**4**(6):e180

[57] Cannarozzi G, Schraudolph NN, Faty M, von Rohr P, Friberg MT, Roth AC, et al. A role for codon order in translation dynamics. *Cell*. 2010;**141**(2):355-367

[58] Kozak M. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell*. 1986;**44**(2):283-292

[59] Presnyak V, Alhusaini N, Chen YH, Martin S, Morris N, Kline N, et al. Codon optimality is a major determinant of mRNA stability. *Cell*. 2015;**160**(6):1111-1124

[60] Karikó K, Muramatsu H, Keller JM, Weissman D. Increased erythropoiesis in mice injected with submicrogram quantities of pseudouridine-containing mRNA encoding erythropoietin. *Molecular Therapy*. 2012;**20**(5):948-953

[61] Jalkanen AL, Coleman SJ, Wilusz J. Determinants and implications of mRNA poly(A) tail size—Does this protein make my tail look big? *Seminars in Cell & Developmental Biology*. 2014;**34**:24-32

[62] Full article: Developing mRNA-vaccine technologies. DOI: 10.4161/rna.22269

[63] Arbuthnot P, Ely A, Bloom K. A convenient method to generate and maintain poly(A)-encoding DNA sequences required for in vitro transcription of mRNA. *BioTechniques*. 2019;**66**(1):37-39

[64] Grier AE, Burleigh S, Sahni J, Clough CA, Cardot V, Choe DC, et al.

pEVL: A linear plasmid for generating mRNA IVT templates with extended encoded poly(A) sequences. *Molecular Therapy - Nucleic Acids*. 2016;**5**(4):e306

[65] Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis | *Biochemical Journal* | Portland Press. Available from: <https://portlandpress.com/biochemj/article-abstract/377/1/159/43138/Size-dependent-internalization-of-particles-via>

[66] Lipid nanoparticles for mRNA delivery | *Nature Reviews Materials*. Available from: <https://www.nature.com/articles/s41578-021-00358-0>

[67] Chou JY, Mansfield BC. Recombinant AAV-directed gene therapy for type I glycogen storage diseases. *Expert Opinion on Biological Therapy*. 2011;**11**(8):1011-1024

[68] Schott JW, Morgan M, Galla M, Schambach A. Viral and synthetic RNA vector technologies and applications. *Molecular Therapy*. 2016;**24**(9):1513-1527

[69] Han G, Noh D, Lee H, Lee S, Kim S, Yoon HY, et al. Advances in mRNA therapeutics for cancer immunotherapy: From modification to delivery. *Advanced Drug Delivery Reviews*. 2023;**199**:114973

[70] Midoux P, Pichon C. Lipid-based mRNA vaccine delivery systems. *Expert Review of Vaccines*. 2015;**14**(2):221-234

[71] mRNA vaccine delivery using lipid nanoparticles. *Therapeutic Delivery*;7(5). DOI: 10.4155/tde-2016-0006

[72] The role of lipid components in lipid nanoparticles for vaccines and gene therapy. *ScienceDirect*. Available from: https://www.sciencedirect.com/science/article/pii/S0169409X22003064?casa_token=heG9n0E6-QoAAAAA:Hg3rjCQq

ja6r0WV3NNlsh6Qws2zYbSi-xwU315d mXq76z4Jy7tgoyQAb6hqQXlmJhJgKBV jdHf8

[73] Granot Y, Peer D. Delivering the right message: Challenges and opportunities in lipid nanoparticles-mediated modified mRNA therapeutics-An innate immune system standpoint. *Seminars in Immunology*. 2017;**34**:68-77

[74] Tseng YC, Xu Z, Guley K, Yuan H, Huang L. Lipid-calcium phosphate nanoparticles for delivery to the lymphatic system and SPECT/CT imaging of lymph node metastases. *Biomaterials*. 2014;**35**(16):4688-4698

[75] Kong B, Kim Y, Kim EH, Suk JS, Yang Y. mRNA: A promising platform for cancer immunotherapy. *Advanced Drug Delivery Reviews*. 2023;**199**:114993

[76] *Frontiers | Development and delivery systems of mRNA vaccines*. DOI: 10.3389/fbioe.2021.718753/full

[77] Reichmuth AM, Oberli MA, Jaklenec A, Langer R, Blankschtein D. mRNA vaccine delivery using lipid nanoparticles. *Therapeutic Delivery*. 2016;**7**(5):319-334. DOI: 10.4155/tde-2016-0006

[78] Xiao Y, Tang Z, Huang X, Chen W, Zhou J, Liu H, et al. Emerging mRNA technologies: Delivery strategies and biomedical applications. *Chemical Society Reviews*. 2022;**51**(10):3828-3845

[79] Fischer D, Li Y, Ahlemeyer B, Krieglstein J, Kissel T. In vitro cytotoxicity testing of polycations: Influence of polymer structure on cell viability and hemolysis. *Biomaterials*. 2003;**24**(7):1121-1131

[80] Persano S, Guevara ML, Li Z, Mai J, Ferrari M, Pompa PP, et al. Lipopolyplex potentiates anti-tumor immunity of

mRNA-based vaccination. *Biomaterials*. 2017;125:81-89

[81] Cationic liposome-mediated RNA transfection. | *PNAS*. DOI: 10.1073/pnas.86.16.6077

[82] Loquai C, Hassel J, Brück P, Derhovanessian E, Cuk K, Lörks V, et al. 549 An RNA-lipoplex (RNA-LPX) vaccine demonstrates strong immunogenicity and promising clinical activity in a phase I trial in cutaneous melanoma patients with no evidence of disease at trial inclusion. *Journal for Immunotherapy of Cancer*. 2021;9(Suppl 2). Available from: https://jitc.bmj.com/content/9/Suppl_2/A579

[83] Better, Faster, Stronger: Accelerating mRNA-Based Immunotherapies With Nanocarriers - Carvalho - 2024 - *WIREs Nanomedicine and Nanobiotechnology* - Wiley Online Library. DOI: 10.1002/wnan.2017

[84] Nucleic acid-based drugs for patients with solid tumours. *Nature Reviews Clinical Oncology*. Available from: <https://www.nature.com/articles/s41571-024-00883-1>

[85] Fu Q, Zhao X, Hu J, Jiao Y, Yan Y, Pan X, et al. mRNA vaccines in the context of cancer treatment: From concept to application. *Journal of Translational Medicine*. 2025;23(1):12

[86] Ndeupen S, Qin Z, Jacobsen S, Bouteau A, Estanbouli H, Igyártó BZ. The mRNA-LNP platform's lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory. *Iscience*. 2021;24(12). [Epub Nov 20, 2021]. Available from: https://www.cell.com/iscience/fulltext/S2589-0042%2821%2901450-4?_returnURL%3Dhttps%3A//linkinghubelsevier.com/retrieve/pii/S2589004221014504%3Fshowall%3Dtrue

[87] Goswami R, Chatzikleanthous D, Lou G, Giusti F, Bonci A, Taccone M, et al. Mannosylation of LNP results in improved potency for self-amplifying RNA (SAM) vaccines. *ACS Infectious Diseases*. 2019;5(9):1546-1558

[88] Douka S, Brandenburg LE, Casadidio C, Walther J, Garcia BBM, Spanholtz J, et al. Lipid nanoparticle-mediated messenger RNA delivery for ex vivo engineering of natural killer cells. *Journal of Controlled Release*. 2023;361:455-469

[89] Linette GP, Carreno BM. Neoantigen vaccines pass the immunogenicity test. *Trends in Molecular Medicine*. 2017;23(10):869-871

[90] Sahay G, Querbes W, Alabi C, Eltoukhy A, Sarkar S, Zurenko C, et al. Efficiency of siRNA delivery by lipid nanoparticles is limited by endocytic recycling. *Nature Biotechnology*. 2013;31(7):653-658

[91] Li J, Wu Y, Xiang J, Wang H, Zhuang Q, Wei T, et al. Fluoroalkane modified cationic polymers for personalized mRNA cancer vaccines. *Chemical Engineering Journal*. 2023;456:140930

[92] Morazán-Fernández D, Mora J, Molina-Mora JA. In silico pipeline to identify tumor-specific antigens for cancer immunotherapy using exome sequencing data. *Phenomics*. 2023;3(2):130-137

[93] Nelson J, Sorensen EW, Mintri S, Rabideau AE, Zheng W, Besin G, et al. Impact of mRNA chemistry and manufacturing process on innate immune activation. *Science Advances*. 2020;6(26):eaaz6893

[94] Chen Z, Meng C, Mai J, Liu Y, Li H, Shen H. An mRNA vaccine elicits

STING-dependent antitumor immune responses. *Acta Pharmaceutica Sinica B*. 2023;**13**(3):1274-1286

[95] Liu G, Lu Y, Thulasi Raman SN, Xu F, Wu Q, Li Z, et al. Nuclear-resident RIG-I senses viral replication inducing antiviral immunity. *Nature Communications*. 2018;**9**(1):3199

[96] Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nature Immunology*. 2004;**5**(7):730-737

[97] Kubara K, Yamazaki K, Miyazaki T, Kondo K, Kurotaki D, Tamura T, et al. Lymph node macrophages drive innate immune responses to enhance the anti-tumor efficacy of mRNA vaccines. *Molecular Therapy*. 2024;**32**(3):704-721

[98] Heine A, Juranek S, Brossart P. Clinical and immunological effects of mRNA vaccines in malignant diseases. *Molecular Cancer*. 2021;**20**(1):52

[99] Linares-Fernández S, Lacroix C, Exposito JY, Verrier B. Tailoring mRNA vaccine to balance innate/adaptive immune response. *Trends in Molecular Medicine*. 2020;**26**(3):311-323

[100] Puleo J, Polyak K. A Darwinian perspective on tumor immune evasion. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*. 2022;**1877**(1):188671

[101] Hattinger E, Scheiblhofer S, Roesler E, Thalhamer T, Thalhamer J, Weiss R. Prophylactic mRNA vaccination against allergy confers long-term memory responses and persistent protection in mice. *Journal of Immunology Research*. 2015;**2015**:1-12

[102] Schlake T, Thess A, Thran M, Jordan I. mRNA as novel technology for passive immunotherapy. *Cellular and Molecular Life Sciences*. 2019;**76**(2):301-328

[103] Lee JH, Sutton HJ, Cottrell CA, Phung I, Ozorowski G, Sewall LM, et al. Long-primed germinal centres with enduring affinity maturation and clonal migration. *Nature*. 2022;**609**(7929):998-1004

[104] Gergen J, Petsch B. mRNA-based vaccines and mode of action. In: Yu D, Petsch B, editors. *mRNA Vaccines*. Cham: Springer International Publishing; 2020. pp. 1-30. DOI: 10.1007/82_2020_230

[105] Barbier AJ, Jiang AY, Zhang P, Wooster R, Anderson DG. The clinical progress of mRNA vaccines and immunotherapies. *Nature Biotechnology*. 2022;**40**(6):840-854

[106] Enokida T, Moreira A, Bhardwaj N. Vaccines for immunoprevention of cancer. *The Journal of Clinical Investigation*. 2021;**131**(9):146956. Available from: <https://www.jci.org/articles/view/146956>

[107] Bird L. mRNA vaccine for treating pancreatic cancer. *Nature Reviews Immunology*. 2023;**23**(7):413-413

[108] Ye Z, Harmon J, Ni W, Li Y, Wich D, Xu Q. The mRNA vaccine revolution: COVID-19 has launched the future of vaccinology. *ACS Nano*. 2023;**17**(16):15231-15253

[109] Lorentzen CL, Haanen JB, Met Ö, Svane IM. Clinical advances and ongoing trials of mRNA vaccines for cancer treatment. *The Lancet Oncology*. 2022;**23**(10):e450-e458

[110] Shi R, Liu X, Wang Y, Pan M, Wang S, Shi L, et al. Long-term

stability and immunogenicity of lipid nanoparticle COVID-19 mRNA vaccine is affected by particle size. *Human Vaccines & Immunotherapeutics*. 2024;**20**(1):2342592

[111] Synthetic messenger RNA-based vaccines: From scorn to hype. Available from: <https://www.mdpi.com/1999-4915/13/2/270>

[112] The many pathways of RNA degradation: Cell. Available from: [https://www.cell.com/cell/fulltext/S0092-8674\(09\)00067-1?returnURL=http://linkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867409000671%3Fshowall%3Dtrue&cc=y](https://www.cell.com/cell/fulltext/S0092-8674(09)00067-1?returnURL=http://linkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867409000671%3Fshowall%3Dtrue&cc=y)

[113] Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—A new era in vaccinology. *Nature Reviews. Drug Discovery*. 2018;**17**(4):261-279. Available from: <https://www.nature.com/articles/nrd.2017.243>

[114] Cheng F, Wang Y, Bai Y, Liang Z, Mao Q, Liu D, et al. Research advances on the stability of mRNA vaccines. *Viruses*. 2023;**15**(3):668

[115] Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by toll-like receptors: The impact of nucleoside modification and the evolutionary origin of RNA. *Immunity*. 2005;**23**(2):165-175. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1074761305002116>

[116] Zhang L, More KR, Ojha A, Jackson CB, Quinlan BD, Li H, et al. Effect of mRNA-LNP components of two globally-marketed COVID-19 vaccines on efficacy and stability. *npj Vaccines*. 2023;**8**(1):1-14

[117] Laila UE, An W, Xu ZX. Emerging prospects of mRNA cancer vaccines: Mechanisms, formulations, and

challenges in cancer immunotherapy. *Frontiers in Immunology*. 2024;**15**:1448489. DOI: 10.3389/fimmu.2024.1448489/full

[118] Mechanism of action of mRNA-based vaccines. *Expert Review of Vaccines*; **16**(9). DOI: 10.1080/14760584.2017.1355245

[119] Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement | *ACS Nano*. DOI: 10.1021/acsnano.1c04996

[120] Sun D, Lu ZR. Structure and function of cationic and ionizable lipids for nucleic acid delivery. *Pharmaceutical Research*. 2023;**40**(1):27-24

[121] Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science*. 2015;**348**(6230):74-80. DOI: 10.1126/science.aaa6204

[122] Rupaimoole R, Calin GA, Lopez-Berestein G, Sood AK. miRNA deregulation in cancer cells and the tumor microenvironment. *Cancer Discovery*. 2016;**6**(3):235-246

[123] Hinshaw DC, Shevde LA. The tumor microenvironment innately modulates cancer progression. *Cancer Research*. 2019;**79**(18):4557-4566

[124] Munn DH, Bronte V. Immune suppressive mechanisms in the tumor microenvironment. *Current Opinion in Immunology*. 2016;**39**:1-6

[125] Palmer CD, Rappaport AR, Davis MJ, Hart MG, Scallan CD, Hong SJ, et al. Individualized, heterologous chimpanzee adenovirus and self-amplifying mRNA neoantigen vaccine for advanced metastatic solid tumors:

Phase 1 trial interim results. *Nature Medicine*. 2022;**28**(8):1619-1629

[126] A TCR-like CAR Promotes Sensitive Antigen Recognition and Controlled T-cell Expansion Upon mRNA Vaccination | Cancer Research Communications | American Association for Cancer Research. Available from: <https://aacrjournals.org/cancerrescommun/article/2/8/827/707902/A-TCR-like-CAR-Promotes-Sensitive-Antigen>

[127] Mpakali A, Stratikos E. The role of antigen processing and presentation in cancer and the efficacy of immune checkpoint inhibitor immunotherapy. *Cancers (Basel)*. 2021;**13**(1):134

[128] Blum JS, Wearsch PA, Cresswell P. Pathways of antigen processing. *Annual Review of Immunology*. 2013;**31**:443-473

[129] Bonilla FA, Oettgen HC. Adaptive immunity. *Journal of Allergy and Clinical Immunology*. 2010;**125**(2, Supplement 2):S33-S40

[130] Rock KL, York IA, Goldberg AL. Post-proteasomal antigen processing for major histocompatibility complex class I presentation. *Nature Immunology*. 2004;**5**(7):670-677

[131] Jensen PE. Recent advances in antigen processing and presentation. *Nature Immunology*. 2007;**8**(10):1041-1048

[132] Blees A, Janulienė D, Hofmann T, Koller N, Schmidt C, Trowitzsch S, et al. Structure of the human MHC-I peptide-loading complex. *Nature*. 2017;**551**(7681):525-528

[133] Seliger B, Maeurer MJ, Ferrone S. Antigen-processing machinery breakdown and tumor growth. *Immunology Today*. 2000;**21**(9):455-464

[134] Zhao X, Wangmo D, Robertson M, Subramanian S. Acquired resistance to immune checkpoint blockade therapies. *Cancers*. 2020;**12**(5):1161

[135] Full article: Modulating antigen processing for cancer immunotherapy. DOI: 10.4161/onci.27568

[136] Ganjun YU, Yanfeng WU, Xuetao CO. Personalized neoantigen vaccines for tumor immunotherapy: A long hard journey to promising land. *Chinese Journal of Cancer Biotherapy*. 2022;**153**:1-10

[137] Kudrin A. Cancer vaccines: What do we need to measure in clinical trials? *Human Vaccines & Immunotherapeutics*. 2014;**10**(11):3236-3240

[138] Caushi JX, Smith KN. Quantifying the anti-tumor immune response in patients receiving immunotherapy. *Discovery Medicine*. 2017;**24**(130):59-68

[139] Imani S, Li X, Chen K, Maghsoudloo M, Jabbarzadeh Kaboli P, Hashemi M, et al. Computational biology and artificial intelligence in mRNA vaccine design for cancer immunotherapy. *Frontiers in Cellular and Infection Microbiology*. 2024;**14**:1501010

[140] McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: Past, present, and the future. *Cell*. 2017;**168**(4):613-628. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0092867417300661>

[141] Jones MR, Schrader KA, Shen Y, Pleasance E, Ch'ng C, Dar N, et al. Response to angiotensin blockade with irbesartan in a patient with metastatic colorectal cancer. *Annals of Oncology*. 2016;**27**(5):801-806

[142] Bevers S, Kooijmans SAA, Velde EV d, Evers MJW, Seghers S,

- Gitz-Francois JJM, et al. mRNA-LNP vaccines tuned for systemic immunization induce strong antitumor immunity by engaging splenic immune cells. *Molecular Therapy*. 2022;**30**(9):3078-3094
- [143] Borer PN, Dengler B, Tinoco I, Uhlenbeck OC. Stability of ribonucleic acid double-stranded helices. *Journal of Molecular Biology*. 1974;**86**(4):843-853
- [144] Thermodynamic parameters for an expanded nearest-neighbor model for formation of RNA duplexes with Watson-Crick Base Pairs | *Biochemistry*. DOI: 10.1021/bi9809425
- [145] Mathews DH. Revolutions in RNA secondary structure prediction. *Journal of Molecular Biology*. 2006;**359**(3):526-532
- [146] RNAstructure: Software for RNA secondary structure prediction and analysis | *BMC Bioinformatics* | Full Text. DOI: 10.1186/1471-2105-11-129
- [147] Lorenz R, Bernhart SH, Höner Zu Siederdisen C, Tafer H, Flamm C, Stadler PF, et al. ViennaRNA Package 2.0. *Algorithms for Molecular Biology*. 2011;**6**(1):26. DOI: 10.1186/1748-7188-6-26
- [148] Mfold web server for nucleic acid folding and hybridization prediction | *Nucleic Acids Research* | Oxford Academic. Available from: <https://academic.oup.com/nar/article/31/13/3406/2904217>
- [149] Incorporating chemical modification constraints into a dynamic programming algorithm for prediction of RNA secondary structure | *PNAS*. DOI: 10.1073/pnas.0401799101
- [150] Sato K, Hamada M. Recent trends in RNA informatics: a review of machine learning and deep learning for RNA secondary structure prediction and RNA drug discovery. *Briefings in Bioinformatics*. 2023;**24**(4):bbad186. DOI: 10.1093/bib/bbad186/7179751
- [151] Zakov S, Goldberg Y, Elhadad M, Ziv-Ukelson M. Rich parameterization improves RNA structure prediction. *Journal of Computational Biology*. Nov 2011:1525-1542. DOI: 10.1089/cmb.2011.0184
- [152] CONTRAfold: RNA secondary structure prediction without physics-based models | *Bioinformatics* | Oxford Academic. Available from: <https://academic.oup.com/bioinformatics/article/22/14/e90/228433>
- [153] Full article: The four ingredients of single-sequence RNA secondary structure prediction. A unifying perspective. DOI: 10.4161/rna.24971
- [154] Pfold: RNA secondary structure prediction using stochastic context-free grammars | *Nucleic Acids Research* | Oxford Academic. Available from: <https://academic.oup.com/nar/article/31/13/3423/2904236>
- [155] A range of complex probabilistic models for RNA secondary structure prediction that includes the nearest-neighbor model and more. Available from: <https://rnajournal.cshlp.org/content/18/2/193>
- [156] Kato Y, Seki H, Kasami T. RNA pseudoknotted structure prediction using stochastic multiple context-free grammar. *IPSIJ Digital Courier*. 2006;**2**:655-664
- [157] Efficient parameter estimation for RNA secondary structure prediction | *Bioinformatics* | Oxford Academic. Available from: <https://academic.oup.com/bioinformatics/article/23/13/i19/237108>

- [158] Akiyama M, Sato K, Sakakibara Y. A max-margin training of RNA secondary structure prediction integrated with the thermodynamic model. *Journal of Bioinformatics and Computational Biology*. 2018;**16**(06):1840025
- [159] Sato K, Akiyama M, Sakakibara Y. RNA secondary structure prediction using deep learning with thermodynamic integration. *Nature Communications*. 2021;**12**(1):941
- [160] IPknot: Fast and accurate prediction of RNA secondary structures with pseudoknots using integer programming | *Bioinformatics* | Oxford Academic. Available from: <https://academic.oup.com/bioinformatics/article/27/13/i85/17877>
- [161] Chen X, Li Y, Umarov R, Gao X, Song L. RNA secondary structure prediction by learning unrolled algorithms. *arXiv*; 2020. Available from: <http://arxiv.org/abs/2002.05810>
- [162] Fu L, Cao Y, Wu J, Peng Q, Nie Q, Xie X. UFold: Fast and accurate RNA secondary structure prediction with deep learning. *Nucleic Acids Research*. 2022;**50**(3):e14
- [163] RNA secondary structure prediction using an ensemble of two-dimensional deep neural networks and transfer learning | *Nature Communications*. Available from: <https://www.nature.com/articles/s41467-019-13395-9>
- [164] Direct Inference of Base-Pairing Probabilities with Neural Networks Improves Prediction of RNA Secondary Structures with Pseudoknots. Available from: <https://www.mdpi.com/2073-4425/13/11/2155>
- [165] Martinović I, Vlašić T, Li Y, Hooi B, Zhang Y, Šikić M. A comparative review of deep learning methods for RNA tertiary structure prediction. *bioRxiv*. 2024:2024.11.27.625779. DOI: 10.1101/2024.11.27.625779v1
- [166] Pearce R, Omenn GS, Zhang Y. De novo RNA tertiary structure prediction at atomic resolution using geometric potentials from deep learning. *BioRxiv*. 2022:2022-2005
- [167] Integrated mRNA sequence optimization using deep learning | *Briefings in Bioinformatics*. Oxford Academic. Available from: <https://academic.oup.com/bib/article/24/1/bbad001/6987657?login=false>
- [168] Algorithm for optimized mRNA design improves stability and immunogenicity. *Nature*. Available from: <https://www.nature.com/articles/s41586-023-06127-z>
- [169] RNA secondary structure prediction using deep learning with thermodynamic integration. *Nature Communications*. Available from: <https://www.nature.com/articles/s41467-021-21194-4>
- [170] Agarwal V, Shendure J. Predicting mRNA abundance directly from genomic sequence using deep convolutional neural networks. *Cell Reports*. 2020;**31**(7):107663
- [171] A 5' UTR language model for decoding untranslated regions of mRNA and function predictions. *Nature Machine Intelligence*. Available from: <https://www.nature.com/articles/s42256-024-00823-9>
- [172] Wekesa JS, Kimwele M. A review of multi-omics data integration through deep learning approaches for disease diagnosis, prognosis, and treatment. *Frontiers in Genetics*. 2023;**14**:1199087. DOI: 10.3389/fgene.2023.1199087/full
- [173] Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA,

Langer R. Engineering precision nanoparticles for drug delivery. *Nature Reviews. Drug Discovery*. 2021;**20**(2):101-124. Available from: <https://www.nature.com/articles/s41573-020-0090-8>

[174] Wang W, Chen K, Jiang T, Wu Y, Wu Z, Ying H, et al. Artificial intelligence-driven rational design of ionizable lipids for mRNA delivery. *Nature Communications*. 2024;**15**(1):10804. Available from: <https://www.nature.com/articles/s41467-024-55072-6>

[175] Wang B, Pei J, Xu S, Liu J, Yu J. Recent advances in mRNA cancer vaccines: Meeting challenges and embracing opportunities. *Frontiers in Immunology*. 2023;**14**:1246682. DOI: 10.3389/fimmu.2023.1246682/full

[176] Zhu Z, Wang Z, Qi G, Mazur N, Yang P, Liu Y. Brain tumor segmentation in MRI with multi-modality spatial information enhancement and boundary shape correction. *Pattern Recognition*. 2024;**153**:110553

[177] Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nature Methods*. 2015;**12**(5):453-457. Available from: <https://www.nature.com/articles/nmeth.3337>

[178] Nagy ML, Juncker-Jensen A, Ovadia B, Smale R, Yamamoto K, William J, et al. Quantitative image profiling of the tumor microenvironment on double stained immunohistochemistry images using deep learning. *Journal of Clinical Oncology*. 26 May 2019. DOI: 10.1200/JCO.2019.37.15_suppl.e14619

[179] TransSea: Hybrid CNN–Transformer With Semantic Awareness

for 3-D Brain Tumor Segmentation | IEEE Journals & Magazine | IEEE Xplore. Available from: <https://ieeexplore.ieee.org/document/10555375>

[180] Harnessing TME depicted by histological images to improve cancer prognosis through a deep learning system: *Cell Reports Medicine* [Internet]. Available from: [https://www.cell.com/cell-reports-medicine/fulltext/S2666-3791\(24\)00205-2?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS2666379124002052%3Fshowall%3Dtrue](https://www.cell.com/cell-reports-medicine/fulltext/S2666-3791(24)00205-2?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS2666379124002052%3Fshowall%3Dtrue)

[181] Cancer biomarkers - Henry - 2012 - *Molecular Oncology* - Wiley Online Library. DOI: 10.1016/j.molonc.2012.01.010

[182] Bai H, Wang Y, Liu H, Lu J. Development of a four-mRNA expression-based prognostic signature for cutaneous melanoma. *Frontiers in Genetics*. 2021;**12**. DOI: 10.3389/fgene.2021.680617/full

[183] Caruso FP, Scala G, Cerulo L, Ceccarelli M. A review of COVID-19 biomarkers and drug targets: Resources and tools. *Briefings in Bioinformatics*. 2021;**22**(2):701-713. Available from: <https://academic.oup.com/bib/article/22/2/701/6024739>

[184] NetMHCpan, a method for quantitative predictions of peptide binding to any HLA-A and -B Locus Protein of Known Sequence | *PLOS One*. DOI: 10.1371/journal.pone.0000796

[185] O'Donnell TJ, Rubinsteyn A, Laserson U. MHCflurry 2.0: Improved pan-allele prediction of MHC class I-presented peptides by incorporating antigen processing. *Cell Systems*. 2020;**11**(1):42-48.e7. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2405471220302398>

- [186] Kim JY, Bang H, Noh SJ, Choi JK. DeepNeo: A webserver for predicting immunogenic neoantigens. *Nucleic Acids Research*. 2023;**51**(W1):W134-W140. Available from: <https://academic.oup.com/nar/article/51/W1/W134/7127215>
- [187] Advances in the development of personalized neoantigen-based therapeutic cancer vaccines | *Nature Reviews Clinical Oncology*. Available from: <https://www.nature.com/articles/s41571-020-00460-2>
- [188] Yang W, Lee KW, Srivastava RM, Kuo F, Krishna C, Chowell D, et al. Immunogenic neoantigens derived from gene fusions stimulate T cell responses. *Nature Medicine*. 2019;**25**(5):767-775
- [189] Turajlic S, Litchfield K, Xu H, Rosenthal R, McGranahan N, Reading JL, et al. Insertion-and-deletion-derived tumour-specific neoantigens and the immunogenic phenotype: A pan-cancer analysis. *The Lancet Oncology*. 2017;**18**(8):1009-1021
- [190] Park J, Chung YJ. Identification of neoantigens derived from alternative splicing and RNA modification. *Genomics Inform*. 2019;**17**(3). DOI: 10.5808/GI.2019.17.3.e23
- [191] Intron retention is a source of neoepitopes in cancer. *Nature Biotechnology*. Available from: <https://www.nature.com/articles/nbt.4239>
- [192] Noncoding regions are the main source of targetable tumor-specific antigens. *Science Translational Medicine*. DOI: 10.1126/scitranslmed.aau5516
- [193] David JK, Maden SK, Weeder BR, Thompson RF, Nellore A. Putatively cancer-specific exon-exon junctions are shared across patients and present in developmental and other non-cancer cells. *NAR Cancer*. 2020;**2**(1):zcaa001
- [194] Halperin RF, Carpten JD, Manojlovic Z, Aldrich J, Keats J, Byron S, et al. A method to reduce ancestry related germline false positives in tumor only somatic variant calling. *BMC Medical Genomics*. 2017;**10**(1):61
- [195] Supernat A, Vidarsson OV, Steen VM, Stokowy T. Comparison of three variant callers for human whole genome sequencing. *Scientific Reports*. 2018;**8**(1):17851
- [196] A machine learning approach for somatic mutation discovery. *Science Translational Medicine*. DOI: 10.1126/scitranslmed.aar7939
- [197] Poplin R, Chang PC, Alexander D, Schwartz S, Colthurst T, Ku A, et al. A universal SNP and small-indel variant caller using deep neural networks. *Nature Biotechnology*. 2018;**36**(10):983-987
- [198] Paul S, Sidney J, Sette A, Peters B. TepiTool: A pipeline for computational prediction of T cell epitope candidates. *Current Protocols in Immunology*. 2016;**114**(1):18.19.1-18.19.24
- [199] Huang PJ, Chang JH, Lin HH, Li YX, Lee CC, Su CT, et al. DeepVariant-on-spark: Small-scale genome analysis using a cloud-based computing framework. *Computational and Mathematical Methods in Medicine*. 2020;**2020**(1):7231205
- [200] Deep convolutional neural networks for accurate somatic mutation detection. *Nature Communications*. Available from: <https://www.nature.com/articles/s41467-019-09027-x>
- [201] Kim JY, Cha H, Kim K, Sung C, An J, Bang H, et al. MHC II immunogenicity shapes the neoepitope landscape in human tumors. *Nature Genetics*. 2023;**55**(2):221-231

- [202] Neoantigens: Promising targets for cancer therapy | Signal Transduction and Targeted Therapy. Available from: <https://www.nature.com/articles/s41392-022-01270-x>
- [203] Murphy JP, Yu Q, Konda P, Paulo JA, Jedrychowski MP, Kowalewski DJ, et al. Multiplexed relative quantitation with isobaric tagging mass spectrometry reveals class I major histocompatibility complex ligand dynamics in response to doxorubicin. *Analytical Chemistry*. 2019;**91**(8):5106-5115
- [204] Melista E, Rigo K, Pasztor A, Christiansen M, Bertinetto FE, Meintjes P, et al. Towards a new gold standard—NGS corrections to sanger SBT genotyping results. *Human Immunology*. 2015;**76**:148
- [205] Sartoris S, Del Pozzo G. Exploring the HLA complex in autoimmunity: From the risk haplotypes to the modulation of expression. *Clinical Immunology*. 2024;**265**:110266
- [206] OptiType: Precision HLA typing from next-generation sequencing data | Bioinformatics | Oxford Academic. Available from: <https://academic.oup.com/bioinformatics/article/30/23/3310/206910>
- [207] Shukla SA, Rooney MS, Rajasagi M, Tiao G, Dixon PM, Lawrence MS, et al. Comprehensive analysis of cancer-associated somatic mutations in class I HLA genes. *Nature Biotechnology*. 2015;**33**(11):1152-1158
- [208] HLA typing: Methods and protocols. SpringerLink. DOI: 10.1007/978-1-4939-8546-3
- [209] Ka S, Lee S, Hong J, Cho Y, Sung J, Kim HN, et al. HLAScan: Genotyping of the HLA region using next-generation sequencing data. *BMC Bioinformatics*. 2017;**18**(1):258
- [210] Reynisson B, Alvarez B, Paul S, Peters B, Nielsen M. NetMHCpan-4.1 and NetMHCIIpan-4.0: Improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. *Nucleic Acids Research*. 2020;**48**(W1):W449-W454
- [211] Marzella DF, Crocioni G, Radusinović T, Lepikhov D, Severin H, Bodor DL, et al. Geometric deep learning improves generalizability of MHC-bound peptide predictions. *Communications Biology*. 2024;**7**(1):1661. Available from: <https://www.nature.com/articles/s42003-024-07292-1>
- [212] Schubert B, Brachvogel HP, Jürges C, Kohlbacher O. EpiToolKit—a web-based workbench for vaccine design. *Bioinformatics*. 2015;**31**(13):2211-2213. Available from: <https://academic.oup.com/bioinformatics/article/31/13/2211/196186>
- [213] Keşmir C, Nussbaum AK, Schild H, Detours V, Brunak S. Prediction of proteasome cleavage motifs by neural networks. *Protein Engineering, Design and Selection*. 2002;**15**(4):287-296. DOI: 10.1093/protein/15.4.287
- [214] Andreatta M, Nielsen M. Gapped sequence alignment using artificial neural networks: Application to the MHC class I system. *Bioinformatics*. 2016;**32**(4):511-517
- [215] Hundal J, Carreno BM, Petti AA, Linette GP, Griffith OL, Mardis ER, et al. pVAC-Seq: A genome-guided in silico approach to identifying tumor neoantigens. *Genome Medicine*. 2016;**8**(1):11. DOI: 10.1186/s13073-016-0264-5

- [216] Lundegaard C, Lamberth K, Harndahl M, Buus S, Lund O, Nielsen M. NetMHC-3.0: Accurate web accessible predictions of human, mouse and monkey MHC class I affinities for peptides of length 8-11. *Nucleic Acids Research*. 2008;**36**(Web Server issue):W509-W512
- [217] Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, Van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnology*. 2010;**28**(5):511-515. Available from: <https://www.nature.com/articles/nbt.1621>
- [218] Khanna A, Larson DE, Srivatsan SN, Mosior M, Abbott TE, Kiwala S, et al. Bam-readcount - rapid generation of basepair-resolution sequence metrics. *ArXiv*. 2021. arXiv:2107.12817v1
- [219] Schubert B, Walzer M, Brachvogel HP, Szolek A, Mohr C, Kohlbacher O. FRED 2: An immunoinformatics framework for python. *Bioinformatics*. 2016;**32**(13):2044-2046. Available from: <https://academic.oup.com/bioinformatics/article/32/13/2044/1743767>
- [220] Bais P, Namburi S, Gatti DM, Zhang X, Chuang JH. CloudNeo: A cloud pipeline for identifying patient-specific tumor neoantigens. *Bioinformatics*. 2017;**33**(19):3110-3112. Available from: <https://academic.oup.com/bioinformatics/article/33/19/3110/3866879>
- [221] Tappeiner E, Finotello F, Charoentong P, Mayer C, Rieder D, Trajanoski Z. TIminer: NGS data mining pipeline for cancer immunology and immunotherapy. *Bioinformatics*. 2017;**33**(19):3140-3141. Available from: <https://academic.oup.com/bioinformatics/article/33/19/3140/3868722>
- [222] Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology*. 2016;**34**(5):525-527. Available from: <https://www.nature.com/articles/nbt.3519>
- [223] Zhang J, Mardis ER, Maher CA. INTEGRATE-neo: a pipeline for personalized gene fusion neoantigen discovery. *Bioinformatics*. 2017;**33**(4):555-557. Available from: <https://academic.oup.com/bioinformatics/article/33/4/555/2623364>
- [224] Tang S, Madhavan S. neoantigenR: An Annotation Based Pipeline for Tumor Neoantigen Identification from Sequencing Data. 2017. preprint. DOI: 10.1101/171843
- [225] Bjerregaard AM, Nielsen M, Hadrup SR, Szallasi Z, Eklund AC. MuPeXI: Prediction of neo-epitopes from tumor sequencing data. *Cancer Immunology, Immunotherapy*. 2017;**66**(9):1123-1130. DOI: 10.1007/s00262-017-2001-3
- [226] Rubinsteyn A, Hodes I, Kodysh J, Hammerbacher J. Vaxrank: A Computational Tool for Designing Personalized Cancer Vaccines. *Europe: PMC*; 2017. DOI: 10.1101/142919
- [227] Wang TY, Wang L, Alam SK, Hoepfner LH, Yang R. ScanNeo: identifying indel-derived neoantigens using RNA-Seq data. *Bioinformatics*. 2019;**35**(20):4159-4161. Available from: <https://academic.oup.com/bioinformatics/article/35/20/4159/5384488>

- [228] McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GRS, Thormann A, et al. The Ensembl variant effect predictor. *Genome Biology*. 2016;**17**(1):122. Available from: <http://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-0974-4>
- [229] Schenck RO, Lakatos E, Gatenbee C, Graham TA, ARA A. NeoPredPipe: High-throughput neoantigen prediction and recognition potential pipeline. *BMC Bioinformatics*. 2019;**20**(1):264. Available from: <https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-019-2876-4>
- [230] Wood MA, Nguyen A, Struck AJ, Ellrott K, Nellore A, Thompson RF. Neoepiscopes improves neoepitope prediction with multivariant phasing. In: Birol I, editor. *Bioinformatics*. Vol. 36. 2020. pp. 713-720. Available from: <https://academic.oup.com/bioinformatics/article/36/3/713/5551338>
- [231] Reynisson B, Barra C, Kaabinejadian S, Hildebrand WH, Peters B, Nielsen M. Improved prediction of MHC II antigen presentation through integration and motif deconvolution of mass spectrometry MHC eluted ligand data. *Journal of Proteome Research*. 2020;**19**(6):2304-2315
- [232] Coelho ACMF, Fonseca AL, Martins DL, Lins PBR, Da Cunha LM, De Souza SJ. neoANT-HILL: An integrated tool for identification of potential neoantigens. *BMC Medical Genomics*. 2020;**13**(1):30. DOI: 10.1186/s12920-020-0694-1
- [233] Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;**29**(1):15-21
- [234] Liao Y, Smyth GK, Shi W. featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*. 2014;**30**(7):923-930. Available from: <https://academic.oup.com/bioinformatics/article/30/7/923/232889>
- [235] Zhang Z, Zhou C, Tang L, Gong Y, Wei Z, Zhang G, et al. ASNEO: Identification of personalized alternative splicing based neoantigens with RNA-seq. *Aging*. 2020;**12**(14):14633-14648. DOI: 10.18632/aging.103516
- [236] Stranzl T, Larsen MV, Lundegaard C, Nielsen M. NetCTLpan: Pan-specific MHC class I pathway epitope predictions. *Immunogenetics*. 2010;**62**(6):357-368
- [237] Leoni G, D'Alise AM, Tucci FG, Micarelli E, Garzia I, De Lucia M, et al. VENUS, a novel selection approach to improve the accuracy of Neoantigens' prediction. *Vaccine*. 2021;**9**(8):880. Available from: <https://www.mdpi.com/2076-393X/9/8/880>
- [238] Wang G, Wan H, Jian X, Li Y, Ouyang J, Tan X, et al. INeo-Epp: A novel T-cell HLA class-I immunogenicity or neoantigenic epitope prediction method based on sequence-related amino acid features. *BioMed Research International*. 2020;**2020**(1):5798356
- [239] Vita R, Overton JA, Greenbaum JA, Ponomarenko J, Clark JD, Cantrell JR, et al. The immune epitope database (IEDB) 3.0. *Nucleic Acids Research*. 2015;**43**(D1):D405-D412
- [240] Chai S, Smith CC, Kocher TK, Hunsucker SA, Beck W, Olsen KS, et al. NeoSplice: a bioinformatics method for prediction of splice variant neoantigens. *Bioinformatics Advances*. 2022;**2**:vbac032. DOI: 10.1093/bioadv/vbac032/6581739

- [241] Li G, Iyer B, Prasath VBS, Ni Y, Salomonis N. DeepImmuno: Deep learning-empowered prediction and generation of immunogenic peptides for T-cell immunity. *Briefings in Bioinformatics*. 2021;22(6):bbab160. DOI: 10.1093/bib/bbab160/6261914
- [242] Montemurro A, Schuster V, Povlsen HR, Bentzen AK, Jurtz V, Chronister WD, et al. NetTCR-2.0 enables accurate prediction of TCR-peptide binding by using paired TCR α and β sequence data. *Communications Biology*. 2021;4(1):1-13
- [243] Deep learning-based prediction of the T cell receptor–antigen binding specificity | *Nature Machine Intelligence*. Available from: <https://www.nature.com/articles/s42256-021-00383-2>
- [244] Kiyotani K, Mai TH, Nakamura Y. Comparison of exome-based HLA class I genotyping tools: Identification of platform-specific genotyping errors. *Journal of Human Genetics*. 2017;62(3):397-405
- [245] Zhou C, Wei Z, Zhang Z, Zhang B, Zhu C, Chen K, et al. pTuneos: Prioritizing tumor neoantigens from next-generation sequencing data. *Genome Medicine*. 2019;11(1):67
- [246] Abstract 3995: pVAC-Seq: A genome-guided in silico approach to identify tumor neoantigens for personalized immunotherapy | *Cancer Research* | American Association for Cancer Research. Available from: https://aacrjournals.org/cancerres/article/76/14_Supplement/3995/611252/Abstract-3995-pVAC-Seq-A-genome-guided-in-silico
- [247] Tang Y, Wang Y, Wang J, Li M, Peng L, Wei G, et al. TruNeo: An integrated pipeline improves personalized true tumor neoantigen identification. *BMC Bioinformatics*. 2020;21(1):532
- [248] DeepImmuno: deep learning-empowered prediction and generation of immunogenic peptides for T-cell immunity | *Briefings in Bioinformatics* | Oxford Academic. Available from: <https://academic.oup.com/bib/article/22/6/bbab160/6261914?login=false>

Oncolytic Virus Therapy: Advancements and Applications in Cancer Care

Asma Ahsan, Saira Dar and Jawad Akhtar

Abstract

Oncolytic viruses (OVs) represent a promising and innovative approach to cancer therapy. These are viruses engineered or naturally selected to specifically infect and destroy cancer cells without harming normal healthy tissue. They work by selectively replicating within and killing tumor cells through direct oncolysis while stimulating the immune system to recognize and attack the tumor. The application of oncolytic viruses is gaining momentum in cancer treatment, supported by a growing body of research and a few clinical successes. Oncolytic viruses work through several key mechanisms e.g., direct tumor cell lysis, immune activation, remodeling of tumor microenvironment (TME), and enhancement of other therapeutic approaches. The current chapter aims to review and summarize the current state-of-the-art information on oncolytic virotherapy.

Keywords: oncolytic viruses, combination therapy, tumor resistance, tumor microenvironment, immunogenic cell death

1. Introduction

Cancers are malignant tumors, that represent a cell mass undergoing uncontrolled cell division driven by mutations in various signaling proteins and pathways [1]. Many anti-cancerous therapeutic implications e.g., radiotherapy, chemotherapy, and immunotherapy are in the clinic with promising outcomes. However, they are still associated with many side effects, damaging healthy cells alongside cancerous cells [2].

One approach to cancer treatment focuses on enhancing the body's immune system to target and destroy cancerous cells. Among the various tools in this strategy, oncolytic viruses are gaining attention. Although viruses are typically considered only associated with causing disease the specificity of viruses can be used to target cancer. These viruses can selectively and efficiently kill cancerous cells (**Figure 1**) [3]. Advances in genetic engineering have enabled us to exploit the cytopathic effects of viruses against tumors, allowing these to be repurposed for therapeutic applications.

To efficiently treat cancer, these viruses should be genetically engineered to minimize their pathogenicity and enhance their specificity in targeting and destroying

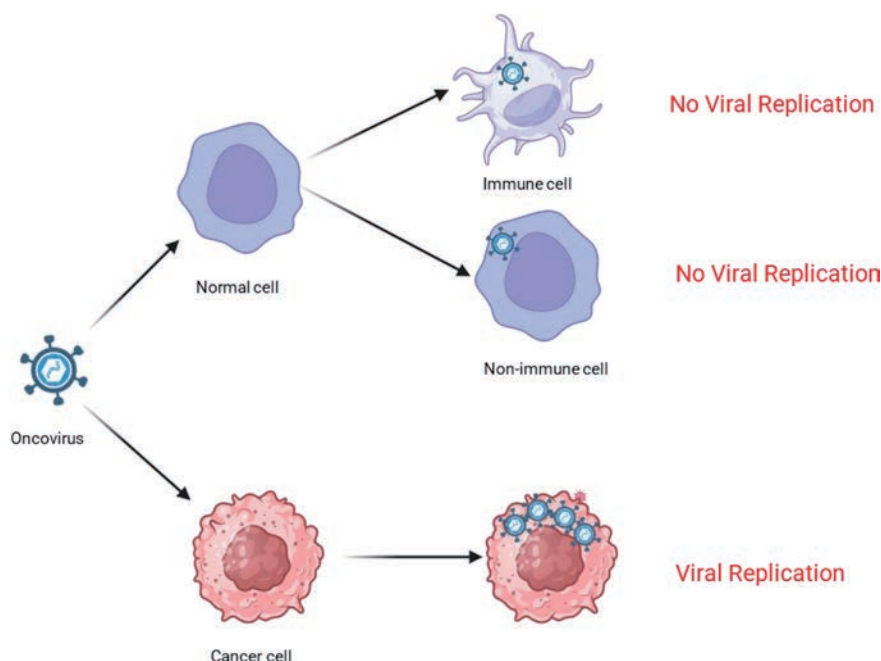


Figure 1.

Selective replication of oncolytic viruses in cancerous cells: Oncolytic viruses can enter both normal and cancer cells due to the presence of specific receptors. However, due to mutated signaling pathways, oncoviruses can replicate exclusively in cancer cells.

tumor cells while ensuring they spare normal cells. But before rendering the viruses to any genetic modification we need a comprehensive understanding of the viral life cycle, mechanisms of cell destruction, and their interactions with the immune system. Moreover, it's important to consider the survival strategies that viruses and cancer cells employ, as well as their potential immune evasion mechanisms, to ensure the virus can successfully fight the tumor [4]. The goal of this review article is to summarize the latest advancements in oncolytic virotherapy.

2. Background

Discovery in science begins with curiosity and observation. The novel approach of using viruses as therapeutic agents has a similarly serendipitous origin. In the eighteenth century, Bohemian anatomist Vaclav Trnka observed the regression of breast cancer in a patient after contracting tertian malaria, which was accompanied by fever [1]. This observation was the first of many reports linking natural pathogenic infections to tumor regression.

The earliest instance of genetic engineering for virus-based cancer treatment occurred in 1991 when Murtaza et al. published their work on modifying Herpes simplex virus-1 to treat mouse glioma tumors. This engineered virus lacks essential proteins to replicate in non-dividing cells [5, 6]. Four years later, an adenovirus carrying a mutated E1B gene (ONYX-015) was tested and demonstrated specific anti-tumor activity with subsequent tumor cell lysis—marking a major milestone in oncolytic virotherapy [7].

In 2004, the first oncolytic virus, RIGVIR, was approved in Latvia for the treatment of melanomas [8]. Since then, significant progress has been made, with two prominent oncolytic viruses receiving approval: H101 in China for nasopharyngeal carcinoma in 2005 [9] and T-VEC in the US and Europe in 2015 [2, 9]. In 2021, Delyact (Tesseraturev/G47Δ), a modified HSV, was approved in Japan for treating malignant myeloma [10, 11].

Current treatment regimens often involve combination therapies, integrating oncolytic viruses with established cancer treatments, such as immunotherapy, chemotherapy and radiotherapy. For example, T-VEC combined with Keytruda is in Phase III clinical trials for the treatment of advanced melanoma [12]. A growing number of oncolytic viruses are in clinical trials across various phases (I, II, and III), marking an exciting era for cancer therapeutics.

3. Mechanism of action

Viruses use diverse mechanisms to invade host cells, replicate, and induce cell lysis. Similarly, oncolytic viruses are tailored to activate unique pathways for selective targeting and infecting tumor cells. The following describes some common mechanisms employed by viruses to eradicate cancer cells.

3.1 Direct lysis of tumor cells

Receptors, which are proteins located on the surface of cells, play a pivotal role in enabling viral entry into the host cell. This step is a prerequisite for viral infection [13]. Viruses exhibit host-specificity i.e., they can gain entry in the cell and become pathogenic, only if their specific receptors are present on the cell surface. For instance, HIV is pathogenic in humans but not chimpanzees. Adenoviruses utilize receptors like integrins, CD46, or the coxsackievirus-adenovirus receptor to enter the host cells [14]. Similarly, the CD46 receptor also plays a role in facilitating the entry of the measles virus. The herpes simplex virus (HSV) utilizes receptors such as NECTIN or Herpesvirus Entry Mediator (HVEM) for successful infection [15].

Once inside the host cell, the virus takes over the cell's metabolic machinery for its benefit. The host cell's proteins are then redirected to support the replication, transcription, and translation of viral components. In normal cells, antiviral signaling pathways are activated to prevent viral replication and infection. A well-known antiviral signaling cascade involves molecules like Janus kinases (JAK), signal transducers and activators of transcription (STAT), and interferon regulatory factor 9 (IRF9), which work together to inhibit viral replication. Cells with these activated pathways are often targeted for cell death through necrosis or apoptosis. Interferon (IFN) produced and released by lysed cells, triggers the expression of IFN receptors and activates an immune response in neighboring cells. This enhanced immune response in uninfected neighboring cells is mediated by proteins such as MYD88, TRIF, IRF3, IRF7, and NF-κB. The production of IFN1 increases the levels of viral-binding proteins, like IRF7, and protein kinases, which help block viral replication in nearby cells. This process creates an immune “hot spot” that limits the spread of the viral infection [16, 17].

Contrarily many antiviral and immune signaling pathways are often impaired in cancerous cells. This allows the virus to survive and replicate within these cells. Viral binding proteins like IRF3 and IRF7 are downregulated in cancer cells, disrupting the IFN1 signaling pathway and weakening the antiviral response. Viral replication

proceeds unchecked without these key components of the antiviral cascade and apoptosis process. This ultimately leads to the lysis (destruction) of the cancer cell, releasing viral particles that can infect and kill neighboring cancer cells, thereby amplifying the therapeutic effect (**Figure 2**) [17].

3.2 Activation of immune response in the tumor microenvironment (TME)

Tumor cell death is not only caused by direct cell lysis but also due to immune system's response. Oncolytic viruses (OVs) activate both innate and adaptive immune responses within the tumor microenvironment [17]. This heightened immune response within the tumor destroys cancer cells, a process known as immunogenic cell death (ICD). Cancer cells undergoing ICD can die through various mechanisms, including apoptosis, necroptosis, and pyroptosis [18].

After cell lysis, tumor cells release pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs include tumor-specific antigens that are released during cell destruction. DAMPs consist of cytokines and proteins such as high mobility group box 1 (HMGB1), mitochondrial DNA (mtDNA), heat shock proteins, ATP, and cytokines like type I interferons (IFNs) and members of the IL-1 family. These DAMPs and PAMPs trigger the release of pro-inflammatory cytokines, such as TNF- α and IL-12, from immune cells, further enhancing the immune response against the tumor [19].

The production of these cytokines activates antigen-presenting cells (APCs), which get mature and begin presenting tumor antigens on their surface. This process

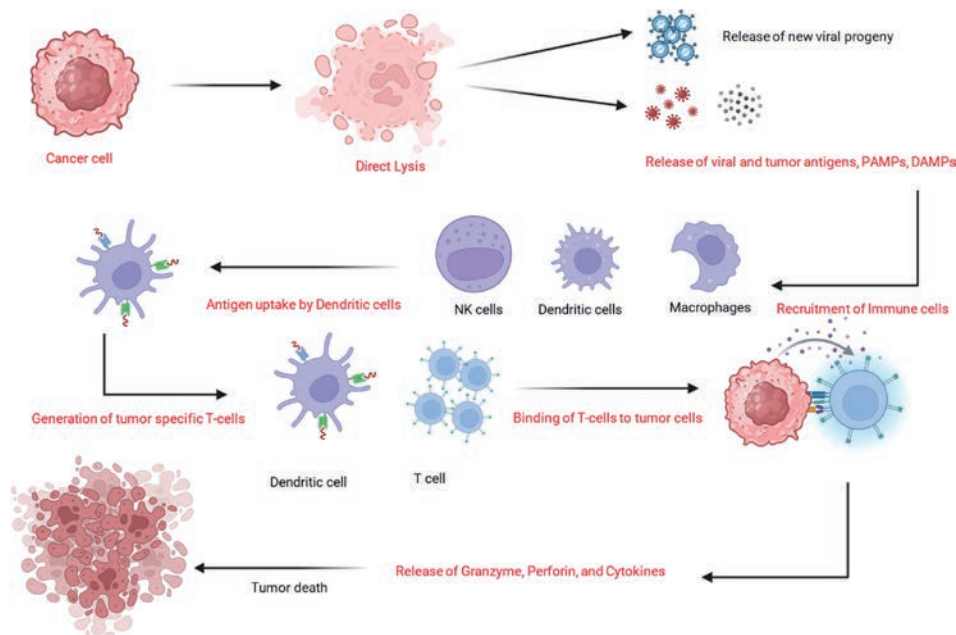


Figure 2.

Fate of cancerous cells after infection with oncolytic viruses: After oncolytic virus infection, cancerous cells undergo direct lysis and release viral and tumor antigens, DAMPs, and PAMPs. These antigens recruit nearby immune cells and trigger an enhanced anti-tumor immune response, which leads to tumor cell death, known as immunogenic cell death.

stimulates the activation and migration of cytotoxic T lymphocytes (CTLs) from primary lymphoid organs to the tumor site. Once at the tumor site, CTLs induce tumor cell lysis by releasing perforins and granzymes. At this stage, the tumor microenvironment is often referred to as a “hot” tumor, due to the heightened immune activity and increased immune cell infiltration [20].

However, virus-infected cancer cells often employ immune evasion strategies, such as reducing the expression of MHC class I molecules on their surface. This reduction in MHC expression makes the cancer cells less visible to cytotoxic T-cells [21]. Natural killer (NK) cells play a crucial role here: they detect the decreased MHC expression and help recruit additional immune cells to the tumor site, further enhancing tumor clearance and contributing to the elimination of cancer [22].

3.3 Modulation of the tumor microenvironment

The vascular system of a tumor plays a vital role in its survival and progression, making it a target for oncolytic viruses (OVs), mediating inhibitory effects on tumor growth. For example, vesicular stomatitis virus (VSV) induces coagulation within tumor blood vessels, leading to vascular collapse [23, 24]. Other OVs are engineered to express the Vstat120 protein, which inhibits endothelial cell migration and tube formation [25]. Treatment of gliomas with OVs expressing Vstat120 resulted in significant suppression of tumor growth, due to the disruption of the tumor vasculature, promoting oncolysis in vitro. The oncolytic vaccinia virus takes a different approach by directly dismantling blood vessels within the tumor. Additionally, some OVs influence tumor neovascularization. For instance, a Vascular endothelial cell growth inhibitor (VEGI) armed oncolytic adenovirus can impact neovascularization and induce mitochondria-mediated cancer cell apoptosis in tumor cells [26], further disrupting the tumor’s blood supply and hindering its growth [27].

4. Types of oncolytic viruses

Different viruses have been genetically engineered to target and trigger an effective anti-tumor response against cancer cells. These oncolytic viruses (OVs) are designed to exploit the unique characteristics of the tumor microenvironment, enabling them to selectively infect and kill tumor cells while minimizing damage to healthy tissue. Genetic modifications can further enhance their therapeutic potential by stimulating robust immune responses and disrupting the tumor’s blood supply. By combining these mechanisms, OVs not only kill the cancer cells but also activate the body’s immune system to sustain anti-tumor activity, ultimately leading to tumor regression and improved therapeutic outcomes.

4.1 Adenovirus

Adenoviruses are particularly well-suited for oncolytic virotherapy because of their ability to infect a wide range of cells (broad tropism). The most commonly utilized types for therapeutic applications are Ad2 and Ad5, which are genetically modified by inducing mutations in the E1B and E2B genes [28]. These mutations help ensure they preferentially infect and replicate within tumor cells while minimizing replication in normal cells.

H101 stands out notably as the first recombinant adenovirus approved for oncolytic therapy [29], especially effective with chemotherapy, overall enhancing the therapeutic effect. Other examples of genetically modified versions are Onyx-015 [30] and CG0070 [31] further demonstrate the therapeutic potential of engineered adenoviruses, with different modifications enhancing their anti-cancer properties.

Additionally, adenoviruses can stimulate the immune response to tumors by antigen presentation to CD8⁺ cytotoxic T cells, enhancing the immune system's ability to recognize and destroy cancer cells more effectively. Taking a step further the VEGI-armed adenovirus targets tumor angiogenesis, reducing the formation of new blood vessels necessary for tumor growth, leading to killing the tumor [29]. Some engineered adenoviruses, like XVir-N-31 [32], are specifically designed to work synergistically with other therapies, such as radiation [33]. This is particularly beneficial in treatments for brain tumors, where targeted therapies that amplify existing treatments (like radiation) can be crucial for improving outcomes.

4.2 Herpes simplex virus (HSV)

Herpes simplex virus (HSV) is a remarkable candidate for oncolytic virotherapy because of its natural ability to cause infections and its adaptability for therapeutic interventions. As an enveloped virus with a double-stranded DNA (dsDNA) genome, HSV has a considerably larger genome than many other viruses, such as adenoviruses [34]. This larger genome allows it to carry more genes and undergo modifications, which are useful for therapeutic applications. Unlike many viruses that require specific receptors to enter host cells, HSV enters the host cell by directly fusing with the host cell's lipid membrane, releasing its genome into the host cytoplasm. Once inside, it replicates its components using host machinery, ultimately causing cell lysis to release new viral progeny. Despite widespread exposure to the antibodies in humans, HSV has evolved various mechanisms to evade the immune system, allowing it to persist in the host [35]. This survival strategy is one of the reasons why HSV is considered most suitable for oncolytic virotherapy—its ability to persist long enough to target and kill cancer cells.

The first oncolytic HSV approved for melanoma treatment, T-VEC, has been engineered to lack the γ 34.5 neurovirulence factor [36]. This modification allows the virus to evade the antiviral responses of normal cells while replicating in tumor cells, which lack robust antiviral defenses. This selective replication ensures that T-VEC specifically targets and kills cancer cells. Another genetically modified HSV strain, G47 Δ is undergoing Phase I/II clinical trials for solid tumors [37]. G47 Δ has been further modified with multiple gene deletions to reduce its susceptibility to be neutralized by the host's immune system.

HSV has also been genetically engineered for other specialized applications. For example, some strains are designed to express epidermal growth factor receptor (EGFR) antibodies like cetuximab [38]. It enables them to target EGFR-positive tumors while promoting immune cell infiltration into tumors improving therapeutic efficacy in animal models. Another approach employs the expression of pro-apoptotic genes (e.g., Her2-COL-sFasL) in HSV, inducing tumor death [39]. Additionally, HSV variants lacking immune checkpoint inhibitors (ICI) like 10PK have demonstrated the ability to enhance the secretion of immune-activating cytokines like TNF- α , GM-CSF, and IL-1 β . These cytokines stimulate robust immune responses against the tumor, further improving their

therapeutic potential [40]. Collectively these advancements highlight HSV's potential as a powerful tool for immune system activation and cancer therapy.

4.3 Vaccinia virus (VV)

Vaccinia virus (VV) is another strong candidate for oncolytic virotherapy, share some similarities with HSV in its therapeutic applications but possesses unique features that enhance its potential for targeting cancer cells [44]. As a member of the orthopoxvirus family, VV has a large double-stranded DNA genome of approximately 190 kb [41], which allows an extensive range of genetic modifications to enhance its potential in cancer therapy. The virus can enter host cells by receptor-mediated endocytosis or by fusion with the host cell membrane under neutral pH conditions [42] providing versatility to target diverse tumor types.

One of the notable features of VV is that it encodes the thymidine kinase enzyme, which is critical for its replication. However, TK expression is low in normal cells and often overexpressed in cancer cells, making it an ideal target for selective replication of the virus in tumor cells. To enhance tumor specificity, VV has been engineered to lack the TK enzyme, preventing its replication in normal cells and restricting replication to cancer cells where TK is overexpressed. A modified strain known as VV JX-594 exemplifies this approach lacking the TK gene, making it a suitable candidate for targeting cancer cells with elevated TK levels [43].

In addition, the VV can be injected intravenously because of its established immune evasion mechanisms that enable it to survive in the bloodstream. The virus's immunogenic proteins are embedded in the viral envelope, preventing the host's antibodies from binding to the virus, therefore avoiding immune clearance. This provides a means to target tumors throughout the body, including those that may be difficult to reach with localized treatments. The VV WR strain is particularly notable for its ability to inhibit the host's antiviral response by blocking interferon receptors. This allows the virus to replicate within infected cells and enhance its oncolytic effects. By inhibiting the host's immune response, VV can spread within the tumor and destroy cancerous cells without being rapidly cleared by the immune system [44].

One strategy to further enhance the oncolytic activity of VV, involves modifying it to induce necroptosis (a form of programmed cell death). In one study, a gene for MLKL, a protein critical for necroptosis, was inserted into the virus. The expression of MLKL promotes increased cell membrane permeabilization, allowing the release of damage-associated molecular patterns (DAMPs), which activate the immune system and further amplify the oncolytic effect [45].

Moreover, VV has been engineered to increase its specificity for certain tumor types by modifying its surface proteins. The EphA2-TEA-VV strain was engineered to express a bispecific T-cell engager (BiTE) that targets the EphA2 receptor, commonly overexpressed in lung cancer cells. This modification enables the virus to activate a direct T-cell-mediated immune response, bypassing the need for traditional antigen presentation, and enhances the therapeutic potential of the virus by directly engaging immune cells to attack the tumor [46].

4.4 Reovirus

Reovirus is a non-enveloped, double-stranded RNA (dsRNA) virus with a genome size of 123 kb. It's naturally found in humans but typically does not cause disease

in healthy individuals. Its dsRNA is crucial for interacting with the host immune system, particularly with protein kinase R (PKR). Reovirus enters host cells through the junctional adhesion molecule A (JAM-A) receptor [47], which is overexpressed in several cancers, including multiple myeloma, non-small cell lung cancer, and diffuse large B-cell lymphoma [48, 49]. This overexpression provides a level of specificity for targeting tumor cells that express JAM-A, making reovirus an ideal candidate for oncolytic virotherapy. In normal cells, PKR binds to the dsRNA of the reovirus, which typically leads to its inactivation [50]. However, in cancer cells, PKR is often inactivated, allowing the virus to continue replicating and ultimately induce cell death. This selective replication in cancer cells without affecting normal cells is a key advantage of reovirus in oncolytic virotherapy [51].

Reolysin is the wild-type reovirus derived from the T3D strain and has been utilized for therapeutic purposes. When administered intravenously, Reolysin targets tumor cells by exploiting the loss of PKR activity in these cells, ensuring continued viral replication and subsequent oncolysis (destruction of cancer cells) [52]. Beyond its cytotoxic effects, the virus also stimulates an anti-tumor immune response, further enhancing its therapeutic potential [56].

5. Challenges with OV as anti-cancerous therapeutic agents

While oncolytic viruses, including reovirus, have shown significant potential in preclinical and clinical studies, their use as a monotherapy against cancer presents several challenges.

5.1 Immune system interference

The host immune system generates antibodies against the virus, which can limit the ability of the virus to reach and effectively eradicate tumors. Furthermore, cytotoxic T lymphocytes (CTLs) and macrophages may also eliminate the virus from infected cells before it can induce any therapeutic effect [53, 54].

5.2 Tumor microenvironment

The dense extracellular matrix (ECM) of tumors consists of collagen and heparan sulfate, which can act as a physical barrier preventing the virus from effectively reaching and penetrating tumors. The heterogeneity of tumor cells further contributes to the challenge, making them unpredictable and more difficult to treat with a one-size-fits-all approach [55].

5.3 Resistance development

Tumors may eventually develop resistance to oncolytic viruses, decreasing their long-term effectiveness. This resistance can manifest as reduced viral replication within the tumor or through the development of immune evasion strategies by the tumor [56].

5.4 Cytokine storms

A potential risk of oncolytic virus therapy is the cytokine storm, characterized by an abnormal overproduction of cytokines. This severe inflammatory response can

lead to severe complications and potentially fatal outcomes, necessitating careful monitoring and management of immune responses during treatment [57].

5.5 Scalability and consistency

Manufacturing oncolytic viruses at a large scale can be challenging, particularly in ensuring consistent viral characteristics throughout the production process. Ensuring consistent immunogenicity and potency is crucial for reliable therapeutic applications.

6. Combined therapy

Combinatorial therapy that integrates oncolytic viruses (OVs) with traditional treatments like radiotherapy and chemotherapy has emerged as an exciting strategy to enhance cancer treatment. This approach leverages the strengths of both OVs and conventional therapies, aims to improve targeted delivery, amplify anti-tumor effects, and increase the overall therapeutic impact. Here's a deeper dive into how these therapies work together and the potential benefits:

6.1 Combination of chemotherapy and OVs

The problem associated with chemotherapy is that alongside targeting rapidly dividing cancer cells it also affects actively dividing normal cells, leading to side effects like hair loss, anemia, nausea, and immune suppression. By combining chemotherapy with OVs, the idea is to harness the strengths of both treatments, using the cytolytic effects of OVs to target cancer cells while complementing the effects of chemotherapy (**Figure 3**) [58].

6.1.1 H101 and chemotherapy

The H101 oncolytic virus derived from adenovirus, was approved in China in 2005 for treating nasopharyngeal carcinoma. This was one of the first examples of an OV being used in combination with chemotherapy. H101 has been shown to enhance tumor response to chemotherapy, likely by improving viral replication and stimulating the immune response. This combination helps to increase tumor-specific killing while mitigating the systemic toxicities of chemotherapy [8]. A study combining oncolytic herpes simplex virus 1 (oHSV1) with mitomycin C (a chemotherapy drug) showed that the combination enhanced tumor sensitivity at various immune checkpoints. This led to a stronger anti-tumor immune response, as the virus's presence helped amplify the effects of the chemotherapy while allowing the immune system to recognize and attack tumor cells [59].

6.1.2 T-VEC and chemotherapy

T-VEC, a modified oncolytic herpes simplex virus, has also been tested along with chemotherapy agents like paclitaxel, doxorubicin, and cyclophosphamide in a Phase II clinical trial targeting triple-negative breast cancer (TNBC). TNBC is a challenging cancer to treat due to its lack of hormone receptors. However, combining T-VEC with chemotherapy has shown potential for enhancing treatment efficacy. This combination may help overcome the limitations of chemotherapy, particularly in more aggressive or advanced stages of TNBC [9].

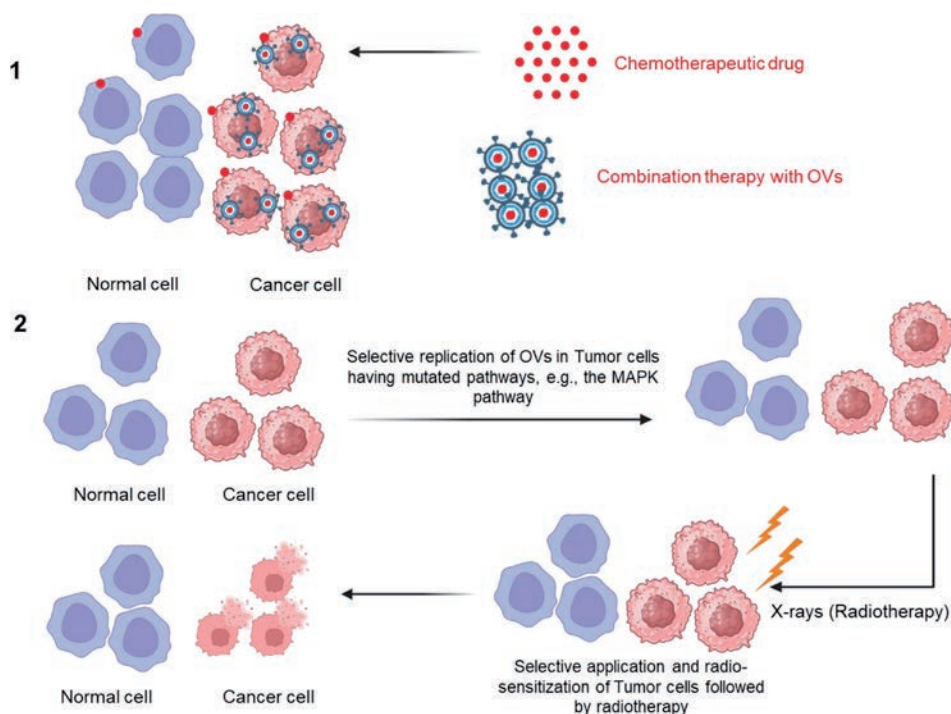


Figure 3. Combinatorial therapy using oncolytic viruses with radiotherapy: Oncolytic viruses exploit mutated signaling pathways, such as the MAPK pathway, to radiosensitize tumor cells. This over-sensitization makes the tumor cells more susceptible to radiotherapy compared to normal cells.

6.2 Combination of radiotherapy and OV's

Combining radiotherapy with oncolytic viruses (OVs) presents a promising approach to cancer treatment, enhancing the effects of radiation while minimizing damage to healthy cells. Radiotherapy employs ionizing radiation to induce DNA damage and generate free radicals, disrupting cellular signaling pathways and ultimately leading to cancer cell death. However, this treatment can also harm surrounding normal tissues, causing significant side effects. By integrating OVs into the treatment regimen, we can enhance tumor-targeting specificity and improve the effectiveness of radiotherapy (**Figure 3**).

Oncolytic viruses can make cancer cells more sensitive to radiation by modulating DNA repair mechanisms. Many OVs have proteins that interact with or target pathways involved in DNA damage response. This interaction inhibits the tumor's ability to repair radiation-induced DNA damage, making the infected cancer cells more vulnerable to the effects of ionizing radiation.

Additionally, OVs can trigger cell death pathways like apoptosis or necrosis. When combined with radiation, these mechanisms may amplify the tumor-killing effect. By sensitizing tumor cells to the DNA damage caused by radiotherapy, OVs increase the likelihood of achieving complete tumor eradication.

One promising example is DNX-2401, an oncolytic adenovirus that has shown promise when used in combination with radiotherapy for the treatment of diffuse intrinsic pontine glioma (DIPG), a highly aggressive and difficult-to-treat brain tumor. DIPG is known for its poor prognosis and resistance to conventional therapies.

In a clinical trial, the combination of DNX-2401 and radiotherapy led to an accelerated anti-tumor T-cell response, contributing to reduced tumor size. The synergy between the oncolytic virus and radiation promoted a more robust immune response, enhancing the overall treatment efficacy [60].

Similarly, XVir-N-31, another oncolytic adenovirus, was combined with radiotherapy to treat brain tumors, demonstrating enhanced lytic effects. The virus not only contributed to tumor cell destruction by replicating and spreading within the tumor but also boosted the efficacy of radiotherapy, leading to a greater therapeutic outcome compared to either treatment alone [32].

Clinical trials with oncolytic viruses like DNX-2401 and XVir-N-31 provide encouraging evidence that combining these therapies can lead to greater therapeutic efficacy and tumor size reduction, especially in challenging cancers like brain tumors. While challenges remain, such as immune clearance of OV and potential safety concerns, this combination therapy represents an exciting frontier in cancer treatment [61].

6.3 Combination of targeted therapy and OV

Combining targeted therapies with oncolytic viruses (OVs) presents an exciting approach to enhance the precision and efficacy of cancer treatment. Targeted therapy aims to selectively target cancer cells thereby minimizing the collateral damage to healthy cells that is common with conventional treatments like chemotherapy and radiotherapy. By combining these therapies with OV, which can directly infect and destroy tumor cells while stimulating an immune response, we can achieve a more targeted, potent, and specific anti-tumor effect. Targeted monoclonal antibodies facilitate virus targeting by directly binding to cancer cell markers. Small molecule inhibitors interfere with the cell signaling pathways within cancer cells. This can help guide the oncolytic virus more precisely to the tumor site. Certain targeted therapies can sensitize tumors to the effects of OV by modifying the tumor microenvironment [61].

In patients with nerve sheath tumors, pretreatment with ruxolitinib (a JAK1/2 inhibitor) enhanced sensitivity to oncolytic herpes simplex virus 1 (oHSV1). Ruxolitinib modulates the immune microenvironment allowing amplified viral replication and increased immune-mediated clearance of the tumor. This combination showed promise in enhancing the therapeutic effects of oHSV1, by enhancing the viral oncolysis and supporting the immune response against the tumor [62].

Oncolytic poxvirus (VACV) has been genetically engineered by deleting the thymidine kinase (TK) gene, which makes the virus more selective for cancer cells [43]. Additionally, PD-1 and 4-1BB antibodies were introduced into the viral genome [63]. Another promising approach involves genetically modified HSV that expresses EGFR antibodies (e.g., cetuximab) [38, 64]. This modification helps enhance immune cell compartmentalization within tumors, increasing immune cell infiltration and thereby improving tumor killing and regression [74].

6.4 Combination of ICI-based therapy and OV

Immune checkpoint inhibitors (ICIs) have become a cornerstone in cancer immunotherapy, harnessing the immune system to kill cancer cells. However, tumors often manipulate their microenvironment to evade immune detection. By blocking the immune checkpoints that tumors exploit to suppress immune responses, ICIs

can restore the immune system's ability to recognize and target these malignant cells. Oncolytic viruses (OVs), which specifically infect and destroy tumor cells while stimulating an immune response, have emerged as powerful agents in combination with ICIs to enhance anti-tumor effects.

The PD-1 protein is expressed on the surface of T-cells. It binds to its ligand PD-L1, which is expressed in tumor cells. This interaction downregulates immune responses, allowing the tumor to evade detection. PD-1 inhibitors block this interaction, keeping the immune system active and capable of recognizing and attacking tumor cells. Drugs such as pembrolizumab, nivolumab, avelumab, atezolizumab, and durvalumab have become widely used in cancer treatment and have shown enhanced protection when combined with OVs. This combination augments immune infiltration within the tumor microenvironment (TME) and boosts anti-tumor immunity.

In a Phase 1/2 clinical trial, DNX-2401, an oncolytic adenovirus, was combined with pembrolizumab, targeting PD-1 [61] and tested in patients suffering from glioblastoma, a highly aggressive brain tumor with a poor prognosis. The combination showed promising results, where pembrolizumab enhanced the immune system's ability to recognize and kill tumor cells, while DNX-2401 infected and killed cancer cells, amplifying the anti-tumor immune response.

A study showed that expressing an anti-PD-L1 antibody in oncolytic herpes simplex virus (oHSV11716) in mice resulted in prolonged survival compared to monotherapy [65]. The anti-PD-L1 antibody led to increased immune cell infiltration within the tumor microenvironment (TME), which enhanced the anti-tumor effect of the oncolytic virus, demonstrating the synergistic potential of combining ICIs and OVs.

CTLA-4 (Cytotoxic T-lymphocyte-associated protein 4) is another immune checkpoint found on T-cells. It binds to CD80/CD86 ligands on antigen-presenting cells (APCs), leading to immune suppression. CTLA-4 inhibitors, such as Ipilimumab, block this interaction, reviving immune responses and allowing for stronger anti-tumor activity. By combining ICIs targeting PD-1 and CTLA-4, a more comprehensive immune activation is achieved, overcoming multiple layers of immune suppression within the tumor. In preclinical models, an oncolytic virus expressing both anti-PD antibodies and anti-CTLA-4 antibodies was able to eradicate glioma tumors in mice [66]. This combination boosted the immune response within the tumor, significantly improving tumor clearance. This suggests that dual immune checkpoint blockade alongside OVs enhances immune system activity within the tumor microenvironment, promoting more effective tumor eradication [67].

A more advanced approach is exploring the triple combination of radiotherapy, oncolytic viruses, and immune checkpoint inhibitors. For instance, combining a PD-L1 inhibitor with GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor) and OVs has shown promising results in clinical trials [68], where this approach increased immune activation and enhanced tumor control. Radiotherapy can damage tumor DNA, creating a favorable environment for OV replication and immune activation, while the PD-L1 blockade further prevents immune evasion by tumors. The synergistic effects observed in preclinical studies and clinical trials underscore the potential for more effective and targeted cancer therapies.

6.5 Combination of adoptive T-cell response therapy with OVs

Adoptive T-cell therapy, particularly with genetically modified T cells, represents a promising frontier in cancer immunotherapy. This approach aims to enhance the immune

system's ability to recognize and attack tumors by harnessing the power of tumor-infiltrating lymphocytes (TILs), chimeric antigen receptor (CAR) T cells, and tumor-specific T-cell receptors (TCRs). Combining these strategies with oncolytic viruses (OVs) offers a synergistic potential to improve the anti-tumor immune response.

TIL therapy is a promising approach where TILs, which naturally infiltrate tumors, are isolated, expanded, and then reinfused into patients to help control tumor growth. TIL therapy has shown substantial success, especially in cancers like ovarian cancer and non-small-cell lung cancer (NSCLC) [69], where these cells can effectively infiltrate and engage with tumor cells. However, activation and antigen presentation issues often limit their effectiveness. Using an oncolytic HSV-1 virus that expresses OX40L and IL-12 has been shown to boost TIL activation and tumor regression in experimental models [67]. OVs like HSV-1 can stimulate immune responses by inducing local inflammation, activating TILs, and enhancing T-cell-mediated tumor killing. TILs can also be used as carriers for targeted OV delivery. Modification of these lymphocytes to specifically recognize tumor antigens can direct OVs more efficiently to tumor sites, improving tumor specificity and viral replication within the tumor.

In TCR therapy, T cells are genetically engineered to express receptors that specifically recognize tumor-associated antigens. By targeting proteins expressed by tumor cells, these modified T cells can more effectively identify and eliminate cancer cells. This approach is further enhanced by combining it with oncolytic viruses (OVs), such as vesicular stomatitis virus (VSV). In this strategy, the oncolytic effect of VSV is directed by using the modified T cells as carriers for the virus, ensuring precise viral delivery and minimizing off-target effects. This method reduces unintended toxicity while maximizing the therapeutic impact on tumor cells [70, 71].

CAR-T cell therapy involves genetically modifying T cells to express chimeric antigen receptors (CARs) that target surface-specific tumor antigens. Kymriah, a CAR-T product approved for acute lymphoblastic leukemia (ALL), involves autologous T cells that are modified to express antigen that targets CD19 receptor, which leads to the elimination of CD19-expressing malignant and normal cells. Additionally, CAR ensures stable and persistent expression of the modified T cells, contributing to the long-term effectiveness of the therapy [72]. The success of Kymriah demonstrates the effectiveness of this approach in liquid tumors.

Solid tumors, such as glioblastoma, often create an immunosuppressive micro-environment that impedes T-cell infiltration. Within tissues, a chemokine gradient governs cell movement, determining which cells can enter the tissues or tumors. In the tumor microenvironment (TME), this gradient is modified to hinder T-cell infiltration. Chemokines such as CXCL9, CXCL11, and CXCL16 play a crucial role in promoting T-cell entry into tumor tissues. In one strategy, T-cell infiltration was enhanced by using a genetically modified oncolytic adenovirus to express the CXCL11 chemokine. This significantly improved the infiltration of CAR-T cells. As a result, more immune cells were recruited, triggering a stronger immune response that ultimately led to tumor regression and clearance [73].

Overall, the combination of OVs with TIL therapy, TCRs, or CAR-T cells can improve tumor specificity [74], boost immune activation, and amplify anti-tumor responses. These approaches also increase immune cell infiltration into the tumor, overcoming some challenges related to tumor immunosuppression and poor immune accessibility. A major challenge with this therapy is T-cell exhaustion. Even genetically modified T cells can experience exhaustion within the tumor, losing their ability to attack cancer cells. ICIs (Immune Checkpoint Inhibitors) may help address this issue when combined with T-cell-based therapies and OVs.

7. Summary

Chemotherapy and radiotherapy, while effective in prolonging survival, cause non-specific cytotoxicity, damaging both healthy and malignant cells. This limitation has spurred interest in oncolytic viruses (OVs), a novel therapeutic class gaining momentum in oncology. OVs are genetically engineered to selectively infect and destroy tumor cells, sparing normal tissues by leveraging the cytolytic properties of viruses designed for tumor specificity.

Genetic modifications of viruses such as Adenovirus, herpes simplex virus (HSV), vaccinia virus, and reovirus enhance their selectivity against cancer cells. Notable examples include RIGVIR (approved in Latvia, 2004), H101 (China, 2005), and talimogene laherparepvec (T-VEC, U.S. and Europe, 2015). Current research explores synergistic combinations of OVs with chemotherapy, radiotherapy, nanomedicine, and immunotherapy to optimize outcomes.

OVs operate via a dual mechanism. They target cancer cells by exploiting defective antiviral defenses—often a result of oncogenic signaling dysregulation—replicating within the host cell to induce lysis and tumor destruction. This process releases tumor-associated antigens and damage-associated molecular patterns (DAMPs), triggering adaptive immunity and amplifying systemic antitumor responses.

Despite their tumor-specific targeting, challenges remain in OV use. Host immunity may clear OVs prematurely, limiting tumor penetration. Tumor resistance can reduce efficacy, while excessive immune activation risks cytokine release syndrome (CRS), a potentially severe inflammatory response. Large-scale production also remains technically demanding. However, combinatorial approaches—particularly with immune checkpoint inhibitors (ICIs) that counteract tumor-induced immunosuppression—demonstrate promising synergy in preclinical and clinical settings, enhancing OV-mediated antitumor activity. For example, T-VEC, an HSV-1 derivative, is approved for melanoma, with other OVs targeting gliomas, breast carcinomas, and more.

By 2020, 97 clinical trials involving 3233 patients reported encouraging results: tumor resistance and immune-mediated OV clearance were manageable in many cases, with minimal severe side effects. Though still evolving, oncolytic virotherapy holds transformative potential. Integration with existing modalities could advance precision oncology, improving efficacy and reducing toxicity compared to conventional therapies, ultimately enhancing patient prognosis and quality of life [75].

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

HSV	herpes simplex virus
OVs	oncoviruses
ICIs	immune checkpoint inhibitors
CAR	chimeric antigen receptor
TILs	T cells, tumor-infiltrating lymphocytes

Author details

Asma Ahsan^{1*}, Saira Dar² and Jawad Akhtar³

1 University of Minnesota, Minneapolis, USA

2 International Islamic University, Islamabad, Pakistan

3 National Institute of Health, USA

*Address all correspondence to: ahsan046@umn.edu

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Brown JS, Amend SR, Austin RH, Gatenby RA, Hammarlund EU, Pienta KJ. Updating the definition of cancer. *Molecular Cancer Research*. 2023;**21**(11):1142-1147
- [2] Yan Z, Zhang Z, Chen Y, Xu J, Wang J, Wang Z. Enhancing cancer therapy: The integration of oncolytic virus therapy with diverse treatments. *Cancer Cell International*. 2024;**24**(1):242
- [3] Hiam-Galvez KJ, Allen BM, Spitzer MH. Systemic immunity in cancer. *Nature Reviews. Cancer*. 2021;**21**(6):345-359
- [4] Bifulco M, Di Zazzo E, Napolitano F, Malfitano AM, Portella G. History of how viruses can fight cancer: From the miraculous healings to the approval of oncolytic viruses. *Biochimie*. 2023;**206**:89-92
- [5] Kelly E, Russell SJ. History of oncolytic viruses: Genesis to genetic engineering. 2007;**15**(4):651-659. Available from: www.moleculartherapy.org
- [6] Ries S, Korn WM. Mini review ONYX-015: Mechanisms of action and clinical potential of a replication-selective adenovirus. *British Journal of Cancer*. 2002;**86**:5-11
- [7] Alberts P, Tilgase A, Rasa A, Bandere K, Venskus D. The advent of oncolytic virotherapy in oncology: The Rigvir® story. *European Journal of Pharmacology*. 2018;**837**:117-126
- [8] Zhang R, Cui Y, Guan X, Jiang X. A recombinant human adenovirus type 5 (H101) combined with chemotherapy for advanced gastric carcinoma: A retrospective cohort study. *Frontiers in Oncology*. 2021;**11**:752504
- [9] Soliman H, Hogue D, Han H, Mooney B, Costa R, Lee MC, et al. Oncolytic T-VEC virotherapy plus neoadjuvant chemotherapy in nonmetastatic triple-negative breast cancer: A phase 2 trial. *Nature Medicine*. 2023;**29**(2):450-457
- [10] Maruyama Y, Sakurai A, Noda S, Fujiwara Y, Okura N, Takagi T, et al. Regulatory issues: PMDA – Review of Sakigake designation products: Oncolytic virus therapy with delytact injection (teserpaturev) for malignant glioma. *Oncologist*. 2023;**28**:664-670
- [11] Frampton JE. Teserpaturev/ G47Δ: First approval. *BioDrugs*. 2022;**36**(5):667-672
- [12] Chesney JA, Ribas A, Long GV, Kirkwood JM, Dummer R, Puzanov I, et al. Randomized, double-blind, placebo-controlled, global phase III trial of talimogene laherparepvec combined with pembrolizumab for advanced melanoma. *Journal of Clinical Oncology*. 2022;**41**:528-540
- [13] Crudden C, Shibano T, Song D, Suleymanova N, Girnita A, Girnita L. Blurring boundaries: Receptor tyrosine kinases as functional G protein-coupled receptors. *International Review of Cell and Molecular Biology*. 2018;**339**:1-40
- [14] Hemsath JR, Liaci AM, Rubin JD, Parrett BJ, Lu SC, Nguyen TV, et al. Ex vivo and In vivo CD46 receptor utilization by species D human adenovirus serotype 26 (HAdV26) VIRUS-CELL INTERACTIONS. *Journal of Virology* jvi.asm.org. 2022;**96**:826-847. Available from: <https://journals.asm.org/journal/jvi>
- [15] Agelidis AM, Shukla D. Cell entry mechanisms of HSV: What we have

learned in recent years. *Future Virology*. 2015;**10**(10):1145-1154

[16] Sever R, Brugge JS. Signal transduction in cancer. Cold Spring Harbor Perspectives in Medicine. 2015;**5**(4):a006098

[17] Chathuranga K, Weerawardhana A, Dodantenna N, Lee JS. Regulation of antiviral innate immune signaling and viral evasion following viral genome sensing. *Experimental and Molecular Medicine*. 2021;**53**(11):1647-1668

[18] Yip HYK, Papa A. Signaling pathways in cancer: Therapeutic targets, combinatorial treatments, and new developments. *Cells*. 2021;**10**(3):1-30

[19] Sun H, Hu W, Yan Y, Zhang Z, Chen Y, Yao X, et al. Using PAMPs and DAMPs as adjuvants in cancer vaccines. *Human Vaccines and Immunotherapeutics*. 2021;**17**(12):5546-5557

[20] Wu B, Zhang B, Li B, Wu H, Jiang M. Cold and hot tumors: From molecular mechanisms to targeted therapy. *Signal Transduction and Targeted Therapy*. 2024;**9**(1):274

[21] Cornel AM, Mimpfen IL, Nierkens S. MHC class I downregulation in cancer: Underlying mechanisms and potential targets for cancer immunotherapy. *Cancers (Basel)*. 2020;**12**(7):1760

[22] Dhatchinamoorthy K, Colbert JD, Rock KL. Cancer immune evasion through loss of MHC class I antigen presentation. *Frontiers in Immunology*. 2021;**12**:636568

[23] Merchan J, Toro BM. Targeting tumor vasculature through oncolytic virotherapy: Recent advances. *Oncolytic Virother*. 2015;**11**(4):169-181

[24] Bishnoi S, Tiwari R, Gupta S, Byraredy SN, Nayak D. Oncotargeting by vesicular stomatitis virus (VSV): Advances in cancer therapy. *Viruses*. 2018;**10**(2):90

[25] Hardcastle J, Kurozumi K, Dmitrieva N, Sayers MP, Ahmad S, Waterman P, et al. Enhanced antitumor efficacy of vasculostatin (Vstat120) expressing oncolytic HSV-1. *Molecular Therapy*. 2010;**18**(2):285-294

[26] Xiao T, Fan JK, Huang HL, Gu JF, Li LY, Liu XY. VEGF-armed oncolytic adenovirus inhibits tumor neovascularization and directly induces mitochondria-mediated cancer cell apoptosis. *Cell Research*. 2010;**20**(3):367-378

[27] Dwivedi S, Purohit P, Mittal Y, Gupta G, Goel A, Verma RC, et al. Genetic engineering: Towards gene therapy and molecular medicine. In: *Omics Technologies and Bio-Engineering: Towards Improving Quality of Life*. Academic Press; 2018. pp. 507-532

[28] Tseha ST. Role of adenoviruses in cancer therapy. *Frontiers in Oncology*. 2022;**12**:772659

[29] Yi L, Ning Z, Xu L, Shen Y, Zhu X, Yu W, et al. The combination treatment of oncolytic adenovirus H101 with nivolumab for refractory advanced hepatocellular carcinoma: An open-label, single-arm, pilot study. *ESMO Open*. 2024;**9**(2):102239

[30] Hu H, Xia Q, Hu J, Wang S. Oncolytic viruses for the treatment of bladder cancer: Advances, challenges, and prospects. *Journal of Clinical Medicine*. 2022;**11**(23):6997

[31] Grandi P, Darilek A, Moscu A, Pradhan A, Li R. Intravesical infusion of oncolytic virus CG0070 in the treatment

of bladder cancer. *Methods in Molecular Biology*. 2023;**2684**:303-317

[32] Schober SJ, Schoening C, Eck J, Middendorf C, Lutsch J, Knoch P, et al. The oncolytic adenovirus XVir-N-31 joins forces with CDK4/6 inhibition augmenting innate and adaptive antitumor immunity in Ewing sarcoma. *Clinical Cancer Research*. 2023;**29**(10):1996-2011

[33] Czolk R, Schwarz N, Koch H, Schötterl S, Wuttke TV, Holm PS, et al. Irradiation enhances the therapeutic effect of the oncolytic adenovirus XVir-N-31 in brain tumor initiating cells. *International Journal of Molecular Medicine*. 2019;**44**(4):1484-1494

[34] Kukhanova MK, Korovina AN, Kochetkov SN. Human herpes simplex virus: Life cycle and development of inhibitors. *Biochemistry (Moscow)*. 2014;**79**(13):1635-1652

[35] Melchjorsen J, Matikainen S, Paludan SR. Activation and evasion of innate antiviral immunity by herpes simplex virus. *Viruses*. 2009;**1**(3):737-759

[36] Bommareddy PK, Patel A, Hossain S, Kaufman HL. Talimogene laherparepvec (T-VEC) and other oncolytic viruses for the treatment of melanoma. *American Journal of Clinical Dermatology*. 2017;**18**(1):1-15

[37] Todo T, Ino Y, Ohtsu H, Shibahara J, Tanaka M. A phase I/II study of triple-mutated oncolytic herpes virus G47Δ in patients with progressive glioblastoma. *Nature Communications*. 2022;**13**(1):4119

[38] Wu Z, Ichinose T, Naoe Y, Matsumura S, Villalobos IB, Eissa IR, et al. Combination of cetuximab and oncolytic virus canerpaturev synergistically inhibits human colorectal

cancer growth. *Molecular Therapy - Oncolytics*. 2019;**13**:107-115

[39] Loya SMW, Zhang X. Enhancing the bystander killing effect of an oncolytic HSV by arming it with a secretable apoptosis activator. *Gene Therapy*. 2015;**22**(3):237-246

[40] Bollino D, Colunga A, Li B, Aurelian L. ΔPK oncolytic activity includes modulation of the tumour cell milieu. *Journal of General Virology*. 2016;**97**(2):496-508

[41] Jacobs BL, Langland JO, Kibler KV, Denzler KL, White SD, Holechek SA, et al. Vaccinia virus vaccines: Past, present and future. *Antiviral Research*. 2009;**84**(1):1-13

[42] Greseth MD, Traktman P. The Life Cycle of the Vaccinia Virus Genome2025. DOI: 10.1146/annurev-virology-091919

[43] Deng L, Fan J, Ding Y, Zhang J, Zhou B, Zhang Y, et al. Oncolytic efficacy of thymidine kinase-deleted vaccinia virus strain Guang9. *Oncotarget*. 2017;**8**:40533-40543. Available from: www.impactjournals.com/oncotarget/

[44] Najarro P, Traktman P, Lewis JA. Vaccinia virus blocks gamma interferon signal transduction: Viral VH1 phosphatase reverses Stat1 activation. *Journal of Virology*. 2001;**75**(7):3185-3196

[45] Van Hoecke L, Riederer S, Saelens X, Sutter G, Rojas JJ. Recombinant viruses delivering the necroptosis mediator MLKL induce a potent antitumor immunity in mice. *Oncoimmunology*. 2020;**9**(1):e1802968

[46] Yu F, Wang X, Guo ZS, Bartlett DL, Gottschalk SM, Song XT. T-cell engager-armed oncolytic vaccinia virus significantly enhances

antitumor therapy. *Molecular Therapy*. 2014;**22**(1):102-111

[47] Magara K, Takasawa A, Osanai M, Ota M, Tagami Y, Ono Y, et al. Elevated expression of JAM-A promotes neoplastic properties of lung adenocarcinoma. *Cancer Science*. 2017;**108**(11):2306-2314

[48] Tian Y, Tian Y, Zhang W, Wei F, Yang J, Luo X, et al. Junctional adhesion molecule-a, an epithelial-mesenchymal transition inducer, correlates with metastasis and poor prognosis in human nasopharyngeal cancer. *Carcinogenesis*. 2015;**36**(1):41-48

[49] Upadhaya P, Barhoi D, Giri A, Bhattacharjee A, Giri S. Joint detection of claudin-1 and junctional adhesion molecule-a as a therapeutic target in oral epithelial dysplasia and oral squamous cell carcinoma. *Journal of Cellular Biochemistry*. 2019;**120**(10):18117-18127

[50] Yue Z, Shatkin AJ. Double-stranded RNA-dependent protein kinase (PKR) is regulated by reovirus structural proteins. *Virology*. 1997;**234**(2):364-371

[51] Fernandes J. Oncogenes: The passport for viral oncolysis through PKR inhibition. *Biomark Cancer*. 2016;**8**:33378

[52] Thompson B. Oncolytics biotech ® Inc.: REOLYSIN ® for melanoma therapy. *Melanoma Management*. 2015;**2**(2):105-107

[53] Seyed-Khorrami SM, Soleimanjahi H, Łos MJ, Zandi K, Emameh RZ. Oncolytic viruses as emerging therapy against cancers including oncovirus-induced cancers. *European Journal of Pharmacology*. 2023;**939**:175393

[54] Raskov H, Orhan A, Christensen JP, Gögenur I. Cytotoxic CD8⁺ T cells in cancer and cancer immunotherapy. *British Journal of Cancer*. 2021;**124**(2):359-367

[55] Henke E, Nandigama R, Ergün S. Extracellular matrix in the tumor microenvironment and its impact on cancer therapy. *Frontiers in Molecular Biosciences*. 2020;**6**:00160

[56] Ambegoda-Liyanage P, Jang SRJ. Resistance in oncolytic viral therapy for solid tumors. *Applied Mathematics and Computation*. 2024;**469**:128546

[57] Dong S, Liu B, Hu S, Guo F, Zhong Y, Cai Q, et al. A novel oncolytic virus induces a regional cytokine storm and safely eliminates malignant ascites of colon cancer. *Cancer Medicine*. 2022;**11**(22):4297-4309

[58] Liu X, Zhang J, Feng K, Wang S, Chen L, Niu S, et al. Efficacy and safety of oncolytic virus combined with chemotherapy or immune checkpoint inhibitors in solid tumor patients: A meta-analysis. *Frontiers in Pharmacology*. 2022;**13**:1023533

[59] Mullerad M, Bochner BH, Adusumilli PS, Bhargava A, Kikuchi E, Hui-Ni C, et al. Herpes simplex virus-based gene therapy enhances the efficacy of mitomycin-C in the treatment of human bladder transitional cell carcinoma. *The Journal of Urology*. 2005;**174**(2):741-746

[60] Nassiri F, Patil V, Yefet LS, Singh O, Liu J, Dang RMA, et al. Oncolytic DNX-2401 virotherapy plus pembrolizumab in recurrent glioblastoma: A phase 1/2 trial. *Nature Medicine*. 2023;**29**(6):1370-1378

[61] Klawitter M, El-Ayoubi A, Buch J, Rüttinger J, Ehrenfeld M, Lichtenegger E, et al. The oncolytic adenovirus XVir-N-31, in combination with the blockade of the PD-1/PD-L1 Axis, conveys abscopal effects in a humanized glioblastoma mouse model. *International Journal of Molecular Sciences*. 2022;**23**(17):9965

- [62] Ghonime MG, Cassady KA. Combination therapy using ruxolitinib and oncolytic HSV renders resistant MPNSTs susceptible to virotherapy. *Cancer Immunology Research*. 2018;**6**(12):1499-1510
- [63] Shi Z, Liu B, Huang C, Xie W, Cen Y, Chen L, et al. An oncolytic vaccinia virus armed with anti-human-PD-1 antibody and anti-human-4-1BB antibody double genes for cancer-targeted therapy. *Biochemical and Biophysical Research Communications*. 2021;**559**:176-182
- [64] Nakano K, Asano R, Tsumoto K, Kwon H, Goins WF, Kumagai I, et al. Herpes simplex virus targeting to the EGF receptor by a gD-specific soluble bridging molecule. *Molecular Therapy*. 2005;**11**(4):617-626
- [65] Streby KA, Geller JI, Currier MA, Warren PS, Racadio JM, Towbin AJ, et al. Intratumoral injection of HSV1716, an oncolytic herpes virus, is safe and shows evidence of immune response and viral replication in young cancer patients. *Clinical Cancer Research*. 2017;**23**(14):3566-3574
- [66] Rotte A. Combination of CTLA-4 and PD-1 blockers for treatment of cancer. *Journal of Experimental and Clinical Cancer Research*. 2019;**38**(1):255
- [67] Liu S, Li F, Ma Q, Du M, Wang H, Zhu Y, et al. OX40L-armed oncolytic virus boosts T-cell response and remodels tumor microenvironment for pancreatic cancer treatment. *Theranostics*. 2023;**13**(12):4016-4029
- [68] Kong Y, Zhao X, Xu M, Pan J, Ma Y, Zou L, et al. PD-1 inhibitor combined with radiotherapy and GM-CSF (PRaG) in patients with metastatic solid tumors: An open-label phase II study. *Frontiers in Immunology*. 2022;**13**:952066
- [69] Kazemi MH, Sadri M, Najafi A, Rahimi A, Baghernejadan Z, Khorramdelazad H, et al. Tumor-infiltrating lymphocytes for treatment of solid tumors: It takes two to tango? *Frontiers in Immunology*. 2022;**13**:1018962
- [70] Hastie E, Grdzlishvili VZ. Vesicular stomatitis virus as a flexible platform for oncolytic virotherapy against cancer. *Journal of General Virology*. 2012;**93**:2529-2545
- [71] Melzer MK, Zeitlinger L, Mall S, Steiger K, Schmid RM, Ebert O, et al. Enhanced safety and efficacy of oncolytic VSV therapy by combination with T cell receptor transgenic T cells as carriers. *Molecular Therapy - Oncolytics*. 2019;**12**:26-40
- [72] Awasthi R, Maier HJ, Zhang J, Lim S. Kymriah® (tisagenlecleucel) – an overview of the clinical development journey of the first approved CAR-T therapy. *Human Vaccines and Immunotherapeutics*. 2023;**19**(1):2210046
- [73] Wang G, Zhang Z, Zhong K, Wang Z, Yang N, Tang X, et al. CXCL11-armed oncolytic adenoviruses enhance CAR-T cell therapeutic efficacy and reprogram tumor microenvironment in glioblastoma. *Molecular Therapy*. 2023;**31**(1):134-153
- [74] Zhu C, Zhao Y, He J, Zhao H, Ni L, Cheng X, et al. TIL-derived CAR T cells improve immune cell infiltration and survival in the treatment of CD19-humanized mouse colorectal cancer. *Cancers (Basel)*. 2023;**15**(23):5567
- [75] Macedo N, Miller DM, Haq R, Kaufman HL. Clinical landscape of oncolytic virus research in 2020. *Journal for Immunotherapy of Cancer*. 2020;**8**(2):e001486

Edited by Thamiris Becker Scheffel

Cancer immunology is transforming the landscape of oncology. This volume explores how the immune system interacts with tumors and how these insights are shaping the future of treatment. From global perspectives, such as the challenges of immunotherapy in Sub-Saharan Africa, to the molecular mechanisms of immune escape and the predictive value of biomarkers, readers are guided through the fundamentals of a rapidly advancing field. The book also examines the tumor microenvironment and the role of the microbiome, revealing their influence on shaping therapy outcomes. Clinical insights emerge through focused discussions on gliomas, melanoma, and liver cancer, demonstrating how immunology translates into therapeutic opportunities. Finally, the volume highlights the frontier of innovation, with chapters on mRNA vaccines and oncolytic viruses pointing toward the next generation of therapies. This collection offers both knowledge and inspiration, showing that cancer immunology is not only a field of research but also a pathway to innovation, resilience, and hope.

Thomas J. FitzGerald, Oncology Series Editor

Published in London, UK

© 2025 IntechOpen
© Jezperklauzen / iStock

IntechOpen

ISSN 3049-8864

ISBN 978-0-85466-326-2

