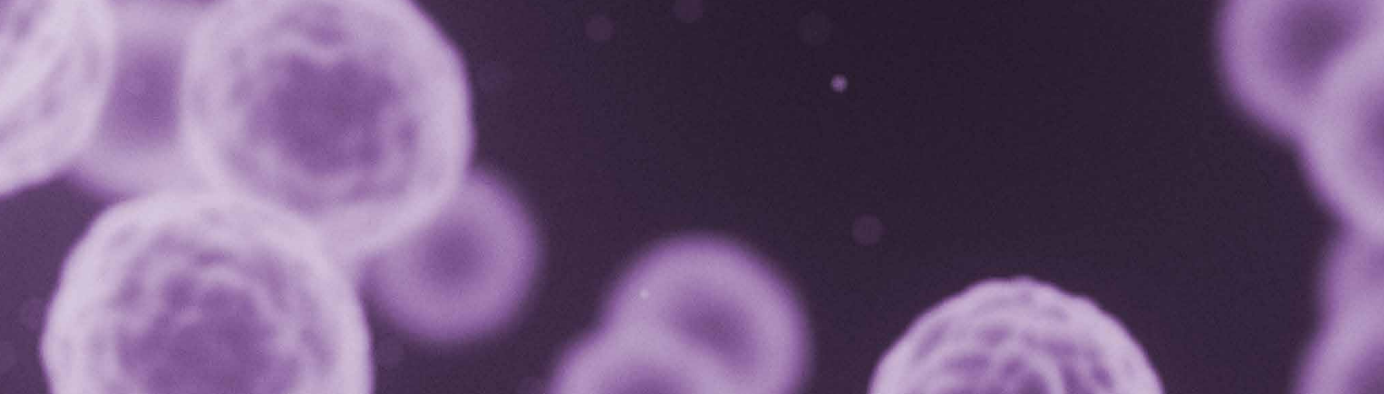




IntechOpen

IntechOpen Series
Oncology, Volume 12

Urologic Cancers

Advances and Challenges in Diagnosis
and Molecular Targeted Therapy

Edited by Soumya Basu and Amit Ranjan



Urologic Cancers - Advances and Challenges in Diagnosis and Molecular Targeted Therapy

Edited by Soumya Basu and Amit Ranjan

Published in London, United Kingdom

Urologic Cancers – Advances and Challenges in Diagnosis and Molecular Targeted Therapy

<http://dx.doi.org/10.5772/intechopen.1004981>

Edited by Soumya Basu and Amit Ranjan

Assistant to the editors: Vishal Kumar Sahu

Contributors

Akshita Saravanan, Amit Ranjan, Aneesa Sultan, Ayesha Rubab, Badr Rafi, Carlos Roberto Jorge Soares, Hassan Fadil, Isabel Heidegger, Kevin Lu, Lujin Alajmani, Marium Jahan, Maryam Fida, Maryam Naeem, Mohsin Ali, Muhammad Zakria, Muhammad Zuhair, Nastasiia Artamonova, Patrícia Falcão, Roshni Matekar, Rowaa A. Banjar, Saanvi Vishnoi, Sabahat Qureshi, Samiksha Bujone, Soumya Basu, Subhayan Sur, Usman Tareen, Vishal Kumar Sahu

© The Editor(s) and the Author(s) 2026

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, 2026 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

Urologic Cancers – Advances and Challenges in Diagnosis and Molecular Targeted Therapy

Edited by Soumya Basu and Amit Ranjan

p. cm.

This title is part of the Oncology Book Series, Volume 12

Series Editor: Thomas J. FitzGerald

Topic: Cancer Diagnostics and Treatment

Topic Editor: Michael Gibson

Print ISBN 978-1-83634-872-6

Online ISBN 978-1-83634-871-9

eBook (PDF) ISBN 978-1-83634-873-3

ISSN 3049-8864

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

IntechOpen Book Series

Oncology

Volume 12

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



Dr. Soumya Basu is an Associate Professor at the Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth (DPU), Pune, India, with over 20 years of experience in cancer biology, molecular signaling, and translational research. Her work addresses key mechanisms underlying tumor progression, metastasis, and therapeutic resistance through integrated experimental, computational, and omics-based approaches. She has published 50+ peer-reviewed research articles, reviews, and book chapters with reputed international publishers. Her current research focuses on small-molecule PPAR- γ partial agonists and AI-driven analysis of exosomal miRNAs in cancer metastasis. Dr. Basu is an active reviewer for international journals, a life member of professional bodies, and contributes to academic leadership, curriculum development, postgraduate mentoring, and industry-academia collaborations.



Dr. Amit Ranjan is an Associate Professor at the Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth (DPU), Pune, India. He has over 19 years of combined teaching and research experience in molecular biology and biotechnology. He earned his M. Sc. from Tezpur University and his Ph.D. from the Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Tata Memorial Centre, HBNI, Mumbai. Dr. Ranjan has qualified for several national competitive examinations, including NET, GATE, and BET. He is a recipient of a travel award and serves as an Innovation Ambassador under the Ministry of Education's Innovation Cell (MIC). His research focuses on the role of glycans in cancer metastasis. He has published 20+ articles in Scopus-indexed journals and contributed four book chapters.

Contents

Preface	XIII
Section 1	
Foundations and Cross-Cutting Horizons in Urologic Cancer	1
Chapter 1	3
Introductory Chapter: An Overview of Urologic Cancers – Molecular Mechanisms and Emerging Therapies <i>by Soumya Basu and Amit Ranjan</i>	
Chapter 2	11
Perspective Chapter: Urologic Cancers – Advances and Challenges in Diagnosis and Molecular Targeted Therapy <i>by Marium Jahan, Mohsin Ali, Maryam Naeem, Muhammad Zakria, Muhammad Zuhair, Ayesha Rubab, Aneesa Sultan, Usman Tareen, Sabahat Qureshi and Maryam Fida</i>	
Section 2	
Prostate Cancer: From Molecular Subtypes to Overcoming Resistance	33
Chapter 3	35
Neuroendocrine Prostate Cancer: An Overlooked Identity? <i>by Nastasiia Artamonova and Isabel Heidegger</i>	
Chapter 4	55
Molecular Mechanisms of Castration Resistance and the Current Clinical Landscape of CRPC Treatment <i>by Soumya Basu, Akshita Saravanan, Roshni Matekar, Samiksha Bujone, Saanvi Vishnoi, Vishal Kumar Sahu, Subhayan Sur and Amit Ranjan</i>	
Chapter 5	83
Production of Human Prostate Adenocarcinoma Tumoroids for Antitumor Testing <i>by Patrícia Falcão and Carlos Roberto Jorge Soares</i>	

Section 3	
Evolving Therapeutic Paradigms in Renal and Urothelial Carcinomas	97
Chapter 6	99
Neoadjuvant Immune Checkpoint Inhibitors and Antibody-Drug Conjugates in Urothelial Carcinoma: Current Evidence and Future Perspectives <i>by Kevin Lu</i>	
Chapter 7	121
Perspective Chapter: Current Role of Adjuvant Therapy in High Risk for Recurrence Resected Kidney Cancer <i>by Lujin Alajmani, Badr Rafi, Hassan Fadil and Rowaa A. Banjar</i>	

Preface

We are pleased to welcome readers to *Urologic Cancers – Advances and Challenges in Diagnosis and Molecular Targeted Therapy*. Within the ever-evolving landscape of oncology, urologic malignancies occupy a particularly important space: one in which discoveries at the molecular level are closely intertwined with urgent clinical demands. This book offers a thoughtful and structured exploration of this field, guiding readers from fundamental biological insights to emerging therapeutic strategies that are redefining patient care.

The primary aim of this book is to present a clear, current, and integrated view of urologic oncology. It is intended as a comprehensive resource for undergraduate students, postgraduate trainees, doctoral researchers, and clinical scientists, helping to build connections between complex molecular processes and their practical translational relevance. By critically examining both progress and persisting limitations, this book aims to cultivate critical thinking, inspire innovative research directions, and contribute meaningfully to the development of future investigators and clinicians.

At a time when precision-driven medicine is reshaping diagnostic and therapeutic decision-making, this book brings together a series of chapters that serve as authoritative guiding references. Collectively, they examine malignancies of the prostate, bladder, kidney, and other genitourinary organs, providing clear insights into underlying disease mechanisms, current diagnostic limitations, and continuously evolving treatment strategies. Each contribution advances understanding within this complex field and supports more informed, effective approaches to patient management in a demanding clinical setting.

This book is organized into three thematic sections, each focusing on a key dimension of urologic cancer.

Section 1, **Foundations and Cross-Cutting Horizons in Urologic Cancer** establishes the book's conceptual framework. This section establishes the book's scope and addresses overarching, cross-cutting themes in urologic cancer. It introduces shared molecular pathways and emerging treatment concepts across urologic cancers, while also addressing overarching technological and methodological challenges. Topics such as biomarker discovery, high-throughput genomic profiling, and advanced computational and data-driven analytical tools are discussed in the context of their growing influence on diagnosis, prognosis, and therapy selection.

Section 2, **Prostate Cancer: From Molecular Subtypes to Overcoming Resistance** focuses on the most prevalent urological malignancy. This section highlights the clinical and biological significance of neuroendocrine prostate cancer, explores the intricate signaling networks underlying castration-resistant prostate cancer, and introduces innovative three-dimensional tumoroid models that are reshaping experimental testing and biomarker development in laboratory settings.

Section 3, **Evolving Therapeutic Paradigms in Renal and Urothelial Carcinomas** addresses the changing standards of care for other major urologic cancers. It offers a critical evaluation of adjuvant therapy in high-risk renal cell carcinoma following surgical intervention and examines the emerging role of neoadjuvant immunotherapeutic strategies and antibody-drug conjugates in urothelial carcinoma, underscoring a shift toward individualized, organ-preserving treatment approaches.

We hope this book succeeds in its goal of disseminating essential, up-to-date knowledge to the academic and scientific community. Beyond serving as an informative reference, it is intended to foster curiosity, encourage interdisciplinary dialogue, and inspire continued innovation in the collective effort to improve outcomes for patients affected by urologic cancers.

Dr. Soumya Basu

Associate Professor,
Cancer and Translational Research Centre,
Dr. D. Y. Patil Biotechnology and Bioinformatics Institute,
Dr. D. Y. Patil Vidyapeeth, Tathawade,
Pune, India

Dr. Amit Ranjan

Associate Professor,
Cancer and Translational Research Centre,
Dr. D. Y. Patil Biotechnology and Bioinformatics Institute,
Dr. D. Y. Patil Vidyapeeth, Tathawade,
Pune, India

Mr. Vishal Kumar Sahu

Senior Research Fellow,
Council of Scientific and Industrial Research,
Govt. of India,
Cancer and Translational Research Centre,
Dr. D. Y. Patil Biotechnology and Bioinformatics Institute,
Dr. D. Y. Patil Vidyapeeth, Tathawade, Pune, India

Dedication

To the clinician and scientists of the past, present, and future whose perseverance, insight, and commitment have driven progress in understanding urologic malignancies;

and to the countless unnamed patients and individuals whose donated tissues and cells—offered in hope, trust, or silence—have enabled advances in urological cancer diagnosis, molecular targeting, and therapeutic innovation.

This book is dedicated in gratitude to those whose lives, known and unknown, continue to illuminate the path toward more precise, compassionate, and effective urological cancer care.

Section 1

Foundations and
Cross-Cutting Horizons
in Urologic Cancer

Introductory Chapter: An Overview of Urologic Cancers – Molecular Mechanisms and Emerging Therapies

Soumya Basu and Amit Ranjan

1. Epidemiology, pathology, and molecular basis of urologic cancers

Urologic cancers represent a diverse spectrum of malignancies within the genitourinary tract, posing a significant global health burden. The most prevalent include prostate, kidney, and bladder cancers, whereas penile, testicular, and urethral cancers are rarer but clinically significant. According to GLOBOCAN 2022, prostate cancer ranks as the second most diagnosed malignancy in men and the fifth leading cause of male cancer-related deaths, with approximately 1,467,854 new cases and 397,430 deaths, while bladder cancer ranks ninth globally with 614,298 cases and 220,596 deaths [1].

Histopathologically, each cancer type displays distinct features: Prostate cancer typically originates in the acinar epithelium of the peripheral zone, with high-grade prostatic intraepithelial neoplasia (HGPIN) acting as a precursor lesion characterized by epithelial proliferation with nuclear atypia and intact basal cells [2, 3]. Renal cell carcinoma (RCC), arising from renal tubular epithelium, can spread *via* the lymphatics or renal vein and is subtyped into clear-cell (von Hippel-Lindau, VHL alterations), papillary (MET or fumarate hydratase, FH alterations), and chromophobe variants (chromosomal and mitochondrial abnormalities) [2, 4]. Urothelial carcinoma (UC) originates in the bladder and ureter's epithelial lining, presenting as non-muscle-invasive (Ta, T1, carcinoma *in situ*) or muscle-invasive forms ($\geq T2$) with metastasis potential [2]. Penile squamous cell carcinoma, though rare, is aggressive and linked to HPV infection, phimosis, and chronic inflammation, with predictable lymphatic spread to the inguinal-femoral regions [5]. Testicular germ-cell tumors such as seminomas and non-seminomas primarily affect young men and respond well to cisplatin-based combination therapy, achieving high cure rates [6]. Urethral carcinoma varies histologically according to site and sex, encompassing urothelial, squamous, or adenocarcinomatous elements (**Figure 1** and **Table 1**) [2].

Molecularly, these malignancies exhibit shared hallmarks of genomic instability and pathway dysregulation. In RCC, VHL loss causes hypoxia-inducible factor (HIF) stabilization, promoting angiogenesis *via* vascular endothelial growth factor/platelet-derived growth factor (VEGF/PDGF) activation. Papillary RCC features MET activation or FH deficiency, while chromophobe RCC shows chromosomal deletions and mitochondrial

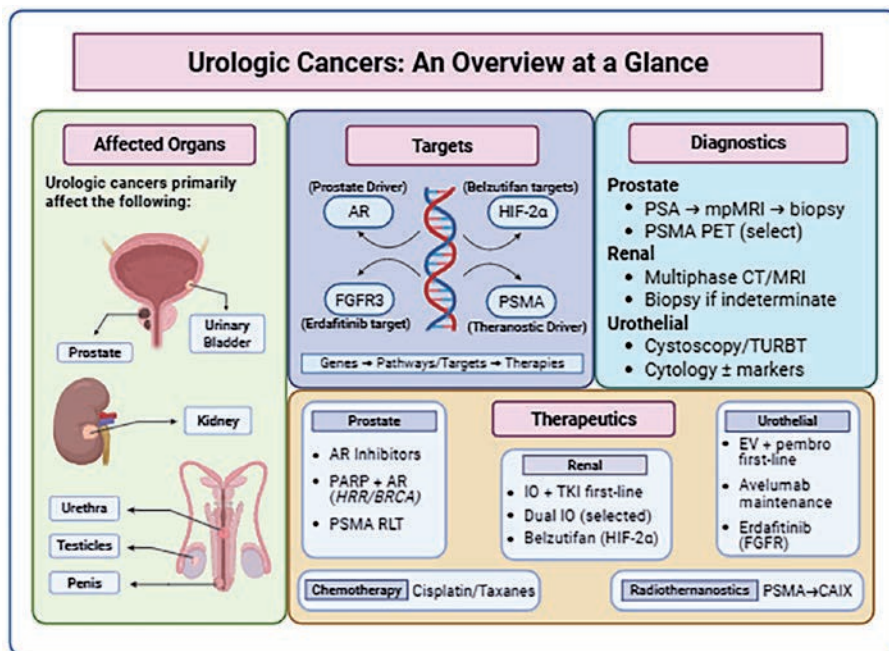


Figure 1.
Overview of different affected organs in urologic cancers, their diagnostics, and therapeutic targets.

defects [2]. In UC, frequent FGFR3 mutations, TERT promoter mutations, and PI3K/AKT, TP53, and RB1 disruptions define the distinct molecular subgroups stratified into luminal and basal/squamous transcriptomic phenotypes [10]. Prostate cancer pathogenesis revolves around androgen receptor (AR) signaling, influenced by alterations such as TMPRSS2–ERG fusions, PTEN loss, and mutations in TP53, SPOP, and DNA-repair genes. Tumors can evolve into AR-independent or neuroendocrine phenotypes under the therapeutic pressure, contributing to treatment resistance [3]. Collectively, molecular and histopathologic integration forms the basis of precision diagnostics and individualized therapeutic approaches in modern urologic oncology.

2. Diagnostics, therapeutics, challenges, and future prospects in precision urologic oncology

The diagnostic assessment of urologic malignancies relies on multimodal integration. In bladder cancer, cystoscopy and transurethral resection of bladder tumor (TURBT) remain the diagnostic cornerstone, supplemented by urine cytology and biomarker assays [10]. Renal tumors are identified through multiphase CT or MRI, while prostate cancer detection employs serum PSA, multiparametric MRI, and targeted or systematic biopsies for risk stratification [3]. Radiological modalities such as CT and MRI are pivotal for staging, and molecular imaging—especially PSMA PET—has transformed prostate cancer management, with novel tracers under evaluation for RCC and UC. Companion diagnostics increasingly guide therapy, such as FGFR2/3 testing in UC or HRR mutation screening in advanced prostate cancer [2].

Tumor type	Red flags	Staging imaging	Companion tests	References
Prostate cancer	Prostate-specific membrane antigen (PSMA) expression elevated; abnormal DRE, bone pain; anemia	MRI to evaluate extra-prostatic extension, lymph node metastasis, and involvement of the seminal vesicle; PET/CT scan	Genetic testing, for example, prostate-specific antigen (HRR)/ (breast cancer gene) BRCA mutations; prostate-specific antigen (PSA) for screening and recurrence monitoring	[3, 7]
Renal carcinoma (kidney)	Flank pain; palpable mass; hematuria; unexplained fever; polycythemia	Cross-sectional imaging (CT) for staging, MRI, and PET imaging	Molecular imaging, for example, PET, carbonic anhydrase IX (CAIX) PET, provides diagnostic and staging information	[4, 8]
Urothelial cancer (bladder)	Gross hematuria; dysuria; frequency and urgency; recurrent urinary infections	Lymph node evaluation; CT urogram	Fibroblast growth factor receptor 2 (FGFR2) testing; urine cytology	[9, 10]
Testicular cancer	Firm testicular mass; heaviness in scrotum; pain due to infarction	CT scan for detecting metastasis and staging	Serum tumor markers like alpha-fetoprotein (AFP) and lactate dehydrogenase (LDH) assist in adjuvant therapy	[6]
Penile SCC	Penile lesions, ulcers, chronic inflammation	MRI/CT scan of pelvis; imaging for evaluation of inguinal-femoral lymph nodes	HPV testing	[5]
Urethral carcinoma	Bloody urethral discharge, palpable urethral lesions, painless hematuria	MRI pelvis for local staging; CT scan for metastasis evaluation	Urine cytology; HPV testing; molecular testing in advanced disease	[1, 2]

Table 1.
Diagnostic pathways and companion tests.

Therapeutic paradigms have evolved alongside molecular discoveries. In prostate cancer, AR pathway inhibitors (abiraterone, enzalutamide, apalutamide) are standard, with PARP inhibitors—including talazoparib with enzalutamide and olaparib with abiraterone—approved for HRR-mutated metastatic castration-resistant prostate cancer [7]. In UC, novel frontline treatments combine antibody-drug conjugates (ADCs) and immunotherapy, notably enfortumab vedotin plus pembrolizumab, while erdafitinib targets FGFR2/3-altered tumors [9]. Advanced RCC therapy prioritizes IO/TKI combinations (pembrolizumab-axitinib), dual-IO regimens (nivolumab-ipilimumab), and belzutifan, a HIF-2 α inhibitor effective in VHL-deficient disease [4]. Cisplatin-based chemotherapy remains crucial for testicular GCTs and penile carcinoma (**Table 2** and **Figure 2**) [5, 6].

Despite substantial progress, challenges persist. Intratumoral heterogeneity drives adaptive resistance, with variable AR activity in prostate cancer, complex angiogenic-immune landscapes in RCC, and PSMA-low lesions undermining radioligand efficacy. Biomarker inconsistency and access inequities further

Tumor type	Dominant targets/pathways	Companion test	Representative systemic therapy	References
Prostate cancer	Androgen receptor (AR) signaling drives tumor growth; HRR mutations (BRCA1/2, ATM) and expression of PSMA for targeted therapy	Germline and somatic HRR/BRCA1/2 mutation testing; PET-CT for staging and treatment eligibility; AR-V7 testing	Androgen deprivation therapy (ADT) as a backbone for advanced stages; androgen receptor pathway inhibitors (ARPIs)—(abiraterone, enzalutamide, apalutamide, darolutamide) to block AR signaling; PARP inhibitors for HRR mutations; PSMA radioligand therapy	[7, 11, 12]
RCC (clear-cell)	VHL/HIF pathway, upregulation of VEGF stimulates angiogenesis; PI3K/AKT/mTOR pathway supports tumor proliferation	CT/MRI for detecting metastasis; radiolabelled PET-CT (girentuximab binds to CAIX); clinical trials for VHL mutations	VEGF- targeted TKI (tyrosine kinase inhibitors) slows tumor growth; ICI-TKI combinations (pembrolizumab, nivolumab); mTOR inhibitors (everolimus)	[4, 8]
Urothelial carcinoma	FGFR3 (RTK) mutations and PI3K/AKT/mTOR pathway enhance tumor proliferation and survival; high Programmed Death –Ligand 1 (PD-L1) expression corresponds to immune evasion	PD-L1IHC expression; FGFR mutation testing; CT-MRI for imaging and staging the local tumor	Platinum-based chemotherapy (cisplatin+gemcitabine); immune checkpoint inhibitors (ICIs) blocking PD-L1 interaction; FGFR inhibitors.	[9, 10]
Testicular GCT	KIT mutation, altered RAS pathway drives tumor growth; miRNA/epigenetic changes influence tumor aggressiveness	Serum tumor markers-AFP, β -hCG are elevated in germ cells; LDH correlates with tumor burden; CT scan for metastatic assessment	Chemotherapy—BEP regimen (bleomycin, etoposide, cisplatin); RPLND surgery at early stage (lymph node involvement)	[6]
Penile SCC	HPV-driven oncogenesis (viral E6/E7 oncoproteins) drives malignant transformation of penile epithelial cells; EGFR overexpression promotes survival and invasiveness of the tumor	HPV DNA testing to detect HPV types in tumor; EGFR testing for targeted therapy; MRI/CT for local tumor and lymph node evaluation	Chemoradiation (cisplatin +5-FU) shrinks tumor; EGFR targeted therapy; surgical excision for localized disease	[5]

Tumor type	Dominant targets/pathways	Companion test	Representative systemic therapy	References
Urethral carcinoma	HPV-associated pathways (E6 and E7 inactivate p53) drives tumor growth; PI3K/AKT/mTOR signaling promotes cell growth and metabolism	Cytourethroscopy with biopsy, MRI of pelvis; HPV testing to detect high-risk HPV DNA	Surgical excision for early stages; chemoradiation (includes cisplatin-based regimens); cisplatin-based chemotherapy for advanced carcinoma	[2]

Table 2.
Urologic cancer biomarkers as therapeutic targets.

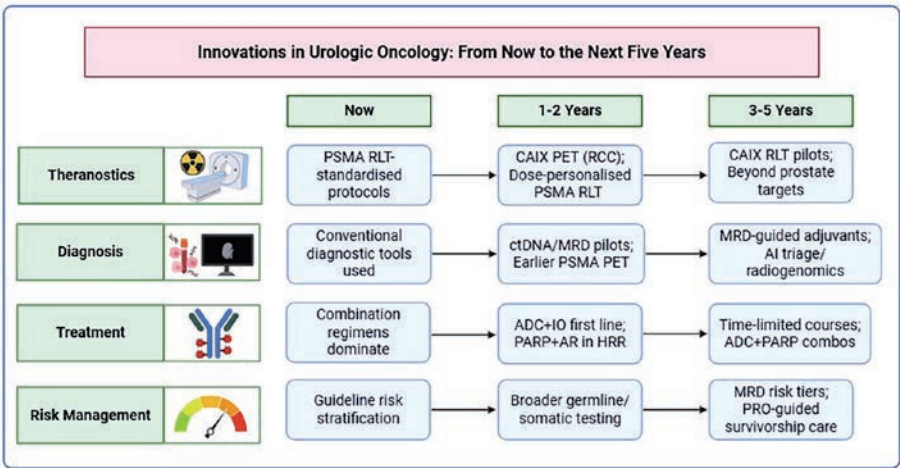


Figure 2.
Future trends in theranostics, diagnosis, treatment, and risk management.

complicate outcomes [4, 12]. Disparities in adopting genomic testing, PSMA PET, and radiopharmaceuticals remain global barriers [11]. Emerging innovations include circulating tumor DNA (ctDNA) and minimal residual disease (MRD) assays for monitoring treatment response and recurrence [13]. Theranostic development continues with CAIX-targeted imaging for RCC and standardized dosimetry for PSMA therapies [8, 11]. Future therapeutic strategies emphasize mechanistically guided combinations—such as ADC + IO, IO + TKI, and PARP + AR inhibitors—and time-limited regimens to balance efficacy with tolerability. Urologic oncology is thus progressing toward an era of biologically driven, accessible, and equitable precision medicine.

Acknowledgements

We express our gratitude to Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Tathawade, Pune, for infrastructural assistance for carrying out this work.

Conflict of interest

The authors declare no conflict of interest.

Author details

Soumya Basu^{*†} and Amit Ranjan^{*†}

Cancer and Translational Research Centre, Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Pune, India

*Address all correspondence to: soumya.bs@gmail.com; soumya.basu@dpu.edu.in and amith.ranjan@dpu.edu.in; bom.amit@gmail.com

† These authors contributed equally.

IntechOpen

© 2026 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] Ferlay J, Ervik M, Lam F, Laversanne M, Colombet M, Mery L, et al. Global Cancer Observatory: Cancer Today. Lyon (FR): International Agency for Research on Cancer; 2024. Available from: <https://gco.iarc.who.int/today> [Accessed: September 5, 2025]
- [2] Gravestock P, Shaw M, Veeratterapillay R, et al. Prostate cancer diagnosis: Biopsy approaches. In: Barber N, Ali A, editors. Urologic Cancers [Internet]. Brisbane (AU): Exon Publications; 12 Sep 2022. Chapter 12. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK585968/doi:10.36255/exon-publications-urologic-cancers-prostate-cancer-biopsy>
- [3] Leslie SW, Soon-Sutton TL, Skelton WP. Prostate cancer. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2025. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK470550/>
- [4] Powles T, Albiges L, Bex A, Comperat E, Grünwald V, Kanesvaran R, et al. Renal cell carcinoma: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Annals of Oncology*. 2024;**35**(8):692-706. DOI: 10.1016/j.annonc.2024.05.537
- [5] Muneer A, Bandini M, Compérat E, De Meerleer G, Fizazi K, Gietema J, et al. Penile cancer: ESMO–EURACAN clinical practice guideline for diagnosis, treatment and follow-up. *ESMO Open*. 2024;**9**(7):103481. DOI: 10.1016/j.esmoop.2024.103481
- [6] Oldenburg J, Berney DM, Bokemeyer C, Climent MA, Cullen M, Dieckmann K-P, et al. Testicular seminoma and non-seminoma: ESMO–EURACAN clinical practice guideline for diagnosis, treatment and follow-up. *Annals of Oncology*. 2022;**33**(4):362-375. DOI: 10.1016/j.annonc.2022.01.002
- [7] Garje R, Bin Riaz I, Naqvi SAA, Rumble RB, Taplin M-E, Kungel TM, et al. Systemic therapy in patients with metastatic castration-resistant prostate cancer: ASCO guideline update. *Journal of Clinical Oncology*. 2025;**43**(20):2311-2333. DOI: 10.1200/JCO-25-00007
- [8] Shuch B, Pantuck AJ, Bernhard JC, Morris MA, Master V, Scott AM, et al. [89Zr]Zr-girentuximab for PET-CT imaging of clear-cell renal cell carcinoma: A prospective, open-label, multicentre, phase 3 trial. *The Lancet Oncology*. 2024;**25**(10):1277-1287. DOI: 10.1016/S1470-2045(24)00402-9
- [9] Powles T, Bellmunt J, Comperat E, De Santis M, Huddart R, Loriot Y, et al. ESMO clinical practice guideline interim update on first-line therapy in advanced urothelial carcinoma. *Annals of Oncology*. 2024;**35**(6):485-490. DOI: 10.1016/j.annonc.2024.03.001
- [10] Raspollini MR, Comperat EM, Lopez-Beltran A, Montironi R, Cimadamore A, Tsuzuki T, et al. News in the classification of WHO 2022 bladder tumors. *Pathologica*. 2023;**115**(1):32. DOI: 10.32074/1591-951X-838
- [11] Kratochwil C, Fendler WP, Eiber M, Hofman MS, Emmett L, Calais J, et al. Joint EANM/SNMMI procedure guideline for the use of ¹⁷⁷Lu-labeled PSMA-targeted radioligand-therapy (¹⁷⁷Lu-PSMA-RLT). *European Journal of Nuclear Medicine and Molecular Imaging*. 2023;**50**(9):2830-2845. DOI: 10.1007/s00259-023-06255-8
- [12] Yu EY, Rumble RB, Agarwal N, Cheng HH, Eggener SE, Bitting RL, et al.

Germline and somatic genomic testing for metastatic prostate cancer: ASCO guideline. *Journal of Clinical Oncology*. 2025;**43**(6):748-758. DOI: 10.1200/JCO-24-02608

[13] Barata PC, Zarrabi KK, Bex A, Grivas P, Hermann K, Hofman MS, et al. Novel methods to assess tumor burden and minimal residual disease in genitourinary cancers. *European Urology*. 2025;**87**(4):412-423. DOI: 10.1016/j.eururo.2024.11.011

Perspective Chapter: Urologic Cancers – Advances and Challenges in Diagnosis and Molecular Targeted Therapy

*Marium Jahan, Mohsin Ali, Maryam Naeem,
Muhammad Zakria, Muhammad Zuhair, Ayesha Rubab,
Aneesa Sultan, Usman Tareen, Sabahat Qureshi
and Maryam Fida*

Abstract

Urologic cancers represent a heterogeneous collection of malignancies of the urinary and male reproductive tracts, including prostate, bladder, adrenal, ureteral, urethral, penile, testicular, and renal cell cancers. This chapter discusses emerging developments and ongoing hurdles in the diagnosis and molecular targeted treatment of these tumors. Special focus is given to the shortcomings of present-day methodologies, such as sub-optimal sensitivity and specificity of available biomarkers and a lack of standardization of liquid biopsy and molecular profiling. In addition, integration of next-generation sequence information in the clinic is hamstrung by the complexity of interpretation and infrastructural requirements. Technological hurdles in artificial intelligence-assisted imaging and pathology make early detection and personalized therapy planning increasingly challenging. By comparing these hurdles with emerging developments, the chapter attempts an overview of present scenarios and future prospects in urologic cancer management.

Keywords: urologic cancers, molecular targeted therapy, diagnostic challenges, liquid biopsy, next-generation sequencing

1. Introduction

1.1 Urologic cancers and their types

Urologic malignancies include cancers that occur in the male reproductive organs and organs of the urinary tract [1]. Urologic malignancies are biologically heterogeneous according to etiology, pathogenesis at the molecular level, and course of therapy. All together, they pose a considerable global health threat, with prostate,

urinary bladder, and kidney cancer among the most frequently diagnosed malignancies worldwide [2].

1.2 Definition and principal body systems impacted

Neoplasms of the urogenital tract, such as the kidneys, ureters, bladder, prostate, testicles, penis, and adrenal glands, are called urologic cancers. The malignancies target the urinary tract and the male reproductive tract [1]. Their etiology depends on the following factors: somatic mutations, environmental factors (tobacco smoking, carcinogens), endocrine control, and chronic inflammatory states. Urologic malignancies potentially disrupt urinary function, endocrine function, and fertility [3].

1.3 Classification of various types

Urologic malignancies are divided based on the anatomical site of origin and histological tumor type. Urothelial carcinoma of the bladder and ureter, adenocarcinoma of the prostate, and clear cell carcinoma of the kidneys are the most common histologies. Staging and grading classifications, like TNM staging and Gleason grading of prostate cancer, are also included for prognosis and treatment [4].

1.3.1 Prostate cancer

Prostate cancer ranks as the second most prevalent cancer in men globally. It arises from glandular epithelial cells of the prostate, most often as adenocarcinoma [5]. Androgen receptor signaling plays a key part in its etiology. Precision therapy has focused on genomic subtyping (e.g., SPOP, ETS-fusion, TP53 mutations) in the latest research. Although many patients have a slow course, advanced disease may transform into castration-resistant types, requiring new androgen receptor-directed agents and PARP inhibitors [6].

1.3.2 Bladder cancer

Bladder cancer occurs most frequently as urothelial carcinoma with strong associations with smoking and occupational exposures [7]. It is notable for its rate of recurrence and progression. Advances have occurred with the identification of molecular subtypes like luminal, basal, and neuronal. These subtypes have implications for prognosis and response to therapy. Immunotherapy, most notably with checkpoint inhibitors such as atezolizumab and pembrolizumab, has transformed the treatment of advanced or metastatic bladder cancer [8].

1.3.3 Renal cancer

Renal cell carcinoma (RCC) represents the most frequent kidney malignancy in adults and accounts for more than 90% of kidney cancer cases. It represents a range of malignancies that include clear cell RCC, papillary RCC, and chromophobe RCC [9]. VHL gene alterations and resultant dysregulation of HIF signaling are the hallmark of clear cell RCC. Molecular profiling has led to the creation of drugs that target VEGF, mTOR, and immune checkpoints and has greatly improved the prognosis of metastatic disease [10].

1.3.4 Testicular cancer

Testicular cancer mainly occurs among young men and is usually defined as germ cell tumors, which are further subdivided into seminomas and non-seminomas. Testicular carcinoma is very treatable, with cisplatin-based chemotherapies serving as the backbone of therapy [11]. New biomarkers like miR-371a-3p have proved useful for diagnosis and disease recurrence monitoring with greater sensitivity and specificity than traditional serum markers (AFP, β -hCG, LDH) [12].

1.3.5 Penile cancer

Penile cancer is an infrequent malignancy involving infection with high-risk human papillomavirus (HPV), usually presented as squamous cell carcinoma. It occurs more frequently in populations where there is limited access for circumcision and HPV vaccine [13]. It can be treated by surgical excision, and in advanced disease, multimodal therapy with radiotherapy and chemotherapy may be of benefit. Molecular research has investigated the p53 mutation and PD-L1 expression in disease development [14].

1.3.6 Urethral cancer

Urethral cancer is a rare and aggressive malignancy that occurs among men and women. It encompasses a range of histologic types like squamous cell carcinoma, adenocarcinoma, and transitional cell carcinoma. Risk factors for the disease are chronic inflammation, history of radiation, and infection by HPV. Owing to its rarity, treatment is patient-specific and usually involves multidisciplinary intervention. Genomic profiling is in its nascent stages but holds promise for new therapies in the future [15].

1.3.7 Ureteral cancer

Ureteral carcinoma, or upper tract cancer of the urinary tract, is most often a carcinoma of the urothelium and has many characteristics of bladder cancer [8]. It may occur as one part of the spectrum of hereditary Lynch syndrome because of germline alterations in DNA mismatch repair genes. Early diagnosis has improved due to advances in ureteroscopy and cytologic sampling, and immune checkpoint inhibition has become available for metastatic disease [16].

1.3.8 Adrenal cancer

Human adrenal cancer, or adrenocortical carcinoma (ACC), occurs rarely but with a poor prognosis. It can be functional (secrete hormones such as adrenocorticoids or androgens) or non-functional [17]. Gene alterations like those of TP53, CTNNB1, and overexpression of IGF2 have been associated with its etiology. The only therapeutic interventions available are surgical removal, mitotane (adrenolytic), and investigational application of molecular-targeting therapies, although the outcomes are limited because of early metastasis and drug resistance [18].

2. Global epidemiology and leading causes

Urologic malignancies account for a high proportion of the incidence and mortality of global cancer, with multiple variations by region and population [2].

The incidence of these malignancies grows continuously due to population aging, changes in lifestyles, environment, and advances in diagnostic techniques. The most prevalent of the five most frequent urologic malignancies, prostate, bladder, kidney, testicular, and penile cancer, have unique epidemiological patterns determined by socioeconomic factors, healthcare access, and hereditary predisposition [19].

2.1 Urologic cancer epidemiological burden

The latest cancer statistics in the world indicate epidemiological peculiarities of urologic cancers according to GLOBOCAN and other registries as of 2024. The most frequently diagnosed male cancer across the globe and particularly in developed countries in Northern and Western Europe, North America, and Australia is prostate cancer due to the extensive screening of PSA and the increased life expectancy [20]. Bladder cancer is number 10 worldwide, and it is more likely to attack men and in areas where there are high levels of smoking and workplace exposures—like Southern and Eastern Europe and North America. Egypt records increased rates owing to squamous cell carcinoma because of schistosomiasis [21]. The rates of kidney cancer are increasing in the modern world, especially in the developed nations, because of obesity and hypertension, with clear cell RCC being the predominant subtype. In low-resource settings, the mortality is still high because of the late detection [22]. Testicular cancer is the most common solid cancer in young men (age 1539) in developed nations, particularly Northern Europe and North America, and its incidence is increasing worldwide [23]. Cancer of the penis and urethra is uncommon but more so in sections of South America, Asia, and Sub-Saharan Africa, where bad hygiene and HPV prevalence are contributing factors [24]. Adrenal cancer (adrenocortical carcinoma) is extremely uncommon (0.722 cases per million per year), late-presenting, and with poor prognosis, with no regional predilection [25].

2.2 Causes of common urologic cancers

Urologic cancers have multifactorial causes that comprise genetic, environmental, infectious, and lifestyle factors. Risk factors of prostate cancer include older age, being of African descent, family history, as well as red meat and dairy-rich diets. Androgen signaling and germline mutations in BRCA1/2 and HOXB13 play important roles [26]. The main risk factors of bladder cancer include tobacco consumption (~50% of cases), exposure to occupational carcinogens (e.g., aromatic amines), chronic cystitis, and infection with *Schistosoma haematobium* in endemic areas [27]. Risk factors of kidney cancer include obesity, hypertension, smoking, exposure to trichloroethylene, and inherited disorders such as von Hippel-Lindau disease [28]. Risk factors of testicular cancer are cryptorchidism, family history, history of testicular tumors, and perhaps endocrine disruptors, and a majority of the tumors develop due to fetal germ cell abnormalities [29]. HPV 16 and 18 are strongly associated with penile cancer as well as poor genital hygiene, absence of circumcision, and chronic inflammation (e.g., lichen sclerosus) [30]. Urethral and ureteral cancers are associated with chronic irritation (e.g., infections, strictures, stones) and frequently accompanied by bladder cancer because of the urothelial common origin [31]. Adrenal cancer is of low prevalence and possibly connected with germline TP53 mutations (e.g., in Li-Fraumeni syndrome) and MEN1; however, its pathogenesis is uncertain [32].

3. Molecular pathogenesis of disease

The molecular context of urologic malignancies is multifaceted and heterogeneous. This is influenced by numerous genomic, epigenetic, transcriptomic, and proteomic changes. Deciphering these underlying molecular drivers has not only elucidated initiation and progression mechanisms but also opened the door for personalized treatment. Multifaceted multi-omics features and single-cell sequencing have revealed key drivers of oncogenesis, mechanisms of drug resistance, and immune evasions in various urologic malignancies [33].

3.1 Genomic, epigenomic, and gene expression

In prostate cancer, driver genomic alterations are TMPRSS2-ERG gene fusion, SPOP, PTEN, and TP53 mutations; BRCA2 and ATM mutations are associated with aggressive disease and sensitivity to PARP inhibitors. GSTP1 methylation and histone modifications are epigenetic effects that affect AR signaling. There are transcriptomic features such as PCA3 and SCHLAP1 that are associated with progression [34].

FGFR3, PIK3CA, TP53, and RB1 mutations are common in bladder cancer, FGFR3 in particular in non-muscle-invasive disease. Early epigenetic silencing of CDKN2A and RASSF1A is also observed. There are differences in the immune profile of the molecular subtypes (luminal-papillary, basal-squamous, neuroendocrine-like) [35].

In renal cell carcinoma (RCC), the loss of VHL leads to HIF-1 α -mediated angiogenesis. Other drivers comprise PBRM1, SETD2, and BAP1. Alterations in epigenetics and gene expression (e.g., CA9, VEGF) are connected with the grade and metastasis of the tumor [36].

NANOG/POU5F1 Pluripotency genes are involved, and i(12p) and KIT mutations characterize testicular cancer. DNA hypomethylation is observed in seminomas, and non-seminomas exhibit varied methylation [37].

Cancer of the penis and urethra frequently deals with HPV, where E6/E7 interfere with p53 and pRb. Urethral cancers present with high mutation loads, with TP53, HRAS, and NOTCH1 mutations [38].

3.2 Signaling pathways and oncogenic drivers

Major oncogenic pathways dysregulated in urologic malignancies are:

Androgen receptor (AR) pathway—A key driver in prostate cancer, AR signaling regulates genes responsible for cell growth. Abnormal AR variants, like AR-V7, contribute to treatment resistance [39].

PI3K/AKT/mTOR axis—Frequently disrupted in prostate, bladder, and kidney cancers due to PTEN loss or PIK3CA mutations. This pathway is targeted by mTOR inhibitors like Everolimus in RCC [40]. FGFR inhibitors such as Erdafitinib have shown clinical effectiveness in FGFR3-mutant bladder cancer [41].

HIF/VEGF pathway—Especially active in clear cell RCC, where VHL gene loss leads to buildup of HIF-1 α . This triggers VEGF and PDGF overexpression and fueling angiogenesis. Drugs like Bevacizumab and Axitinib aim to disrupt this process [42].

Wnt/ β -catenin signaling—Activated in certain urothelial and renal cancers, it plays a role in maintaining cancer stem cell traits and helping tumors evade immune detection [43].

TP53 and RB pathways—Frequently mutated in aggressive bladder and prostate cancers, leading to genomic instability and uncontrolled cell division [44].

Immune checkpoint pathways—Upregulation of PD-L1 and malfunctioning antigen presentation allow advanced RCC and bladder tumors to escape immune attacks. This supports the use of checkpoint inhibitors [45].

4. Advances in diagnostic techniques

The diagnostic approach to urologic cancers has evolved dramatically with the adoption of advanced molecular diagnostics and AI [46]. These tools go beyond standard blood tests and imaging. They offer earlier, more precise, and personalized diagnoses. This progress supports improved risk assessment, continuous monitoring, and customized treatment plans.

4.1 Biomarkers for early detection and risk stratification

In urologic cancers, biomarkers play an important role in diagnosis, monitoring, and treating the disease. PSA is utilized in most cases of prostate cancer, although more accurate tests have been developed (PHI, 4Kscore, SelectMDx) as well as urinary markers (PCA3, TMPRSS2-ERG) [47]. NMP22 and UroVysion FISH are used in the monitoring of bladder cancer, and novel markers such as TERT, FGFR3 mutations, and AURKA are promising [48]. CAIX, KIM-1, and miRNAs (miR-210, miR-1233) are being investigated in renal cell carcinoma [49]. miR-371a-3p is superior to conventional markers (AFP, -hCG, LDH) in the case of testicular cancer [50]. Genomic tools (Oncotype DX, Prolaris, Decipher) can assist with prostate cancer risk stratification [51].

4.2 Non-invasive detection by liquid biopsy

Liquid biopsies are a non-invasive method to detect and monitor tumors [52], ctDNA mutations (e.g., FGFR3, AR-V7) can offer resistance insights [53], CTCs can help prognose metastatic cancers [54], urinary exosomes can aid the diagnostics of prostate and RCC [55], and circulating miRNAs (e.g., miR-21, miR-141) are emerging as promising biomarkers [56].

4.3 Detection with the assistance of imaging methods

Imaging advancements have enhanced tumor detection, staging, and localization through tools like mpMRI for prostate cancer [57], PSMA-PET/CT for precise metastasis detection [58], CECT for RCC and bladder cancer staging [59], CEUS for renal mass characterization [60], and molecular imaging with targeted radio-tracers in RCC [61].

4.4 Molecular profiling by NGS (genomic and transcriptomic)

Next-generation sequencing (NGS) is central to precision oncology, revealing actionable mutations such as FGFR3, PIK3CA, and ERBB2 in bladder cancer [62], VHL and SETD2 in RCC [63], and BRCA2, ATM, and MSH2 in prostate cancer [64], while RNA-seq further aids classification through gene expression profiling and fusion transcript detection like TMPRSS2-ERG [65].

4.5 AI and ML-based diagnosis

Artificial intelligence (AI) and machine learning (ML) are revolutionizing cancer diagnosis by enhancing radiologic analysis [66], automating digital pathology assessments [67], and enabling predictive modeling of outcomes using integrated clinical and molecular data, as seen with FDA-approved tools like ArteraAI [68].

Decision support systems: AI-driven tools are increasingly helping doctors by merging imaging, pathology, and molecular data to generate actionable, patient-specific insights [69].

5. Challenges in diagnostic methods

The current diagnostic methods, despite being advanced and guiding accurate disease diagnosis, face multiple challenges. These challenges are mostly encountered when things come to the practical applicability of these methods.

5.1 Limited sensitivity and specificity of biomarkers

Such biomarkers as prostate-specific antigen (PSA) in prostate cancer or nuclear matrix protein 22 (NMP22) in bladder cancer have long been used to manage urologic cancer, but both have serious limitations because of poor sensitivity and specificity [70]. Although PSA is widely utilized, it can give a false-positive result because of noncancerous conditions such as hyperplasia or prostatitis, and it can fail to detect aggressive cancer when its level of PSA is low, resulting in a delay of diagnosis [71]. More complex biomarkers like lncRNA PCA3 and TMPRSS2\ERG fusion transcripts are still investigational and need specific assays; even then, they do not have adequate standalone accuracy, and multiple markers need to be used together to achieve satisfactory sensitivity and specificity [71, 72]. Urinary protein markers have a better sensitivity, but lower specificity than cytology in bladder cancer, which is also limited in the detection of low-grade tumors. Although Bladder Tumor Antigen is helpful in monitoring, it needs to be used in combination with cystoscopy, and NMP22 is likely to give false positives with benign diseases such as infections, so it cannot be used alone as a diagnostic tool [73].

5.2 Lack of standardization in liquid biopsy and molecular profiling

Liquid biopsies (LBs), consisting of circulating tumor cells, cell-free DNA, and additional tumor-derived elements in bodily fluids, offer a minimally invasive approach to diagnosing and monitoring cancer [74]. Their clinical use in urologic oncology is hampered, though, by the absence of uniform standards among the techniques and tumor types, and thus hampers regulatory approval and reliability [75]. There are methodological issues, such as poor consistency between methods and non-tumor genomic contamination, which can swamp out tumor signals, as well as a lack of well-designed clinical trials to support daily clinical use [75]. ctDNA detection is further undermined by pre-analytical factors including leukocyte lysis in samples that have not been stored properly. Further, the continuing investigation of LBs in urologic malignancies is still restricted by the absence of optimal, standardized detection strategies of biomarkers [74].

5.3 High cost and infrastructure requirements

The current gold standard for the diagnosis of bladder cancer is cystoscopy. This procedure is undoubtedly effective but expensive and places a significant financial burden on the healthcare system [76]. The high cost associated with repeated cystoscopies for bladder cancer surveillance demands the increasing need for more cost-effective diagnostic alternatives. These can include advanced imaging techniques or reliable biomarkers, provided that these alternatives can offer comparable or superior diagnostic accuracy [77].

Certain urinary biomarkers are associated with high costs due to the requirements for specialized laboratory equipment and the need for trained personnel to perform and interpret the tests despite their potential clinical usage [78]. For example, the ImmunoCyt assay demonstrated improved sensitivity for low-grade tumors but requires specific laboratory infrastructure and experienced test readers. A FISH-based assay like UroVysion requires laboratory equipment, skilled interpreters, and a time-consuming specimen processing procedure. Even some non-FDA-approved but promising biomarkers like EpiCheck also have the disadvantage of being costly [79].

The financial implications of routinely using these biomarker tests can be a barrier to their widespread clinical application. This can be the case if their cost is not offset by significant improvements in diagnostic accuracy or a reduction in the need for more expensive procedures.

5.4 Interpretation complexity and clinical integration of NGS data

The interpretation of next-generation sequencing (NGS) reports in clinical oncology is complicated by design and usually requires a joint effort of molecular pathologists, tumor biologists, and clinicians [80]. The fast-track adoption of NGS has placed burdens on data processing, storage, quality control, and interpretation, which require well-developed bioinformatics pipelines and expertise [81]. However, despite the fact that precision oncology is based on more extensive molecular characterization to inform treatment decision-making and enhance patient outcomes, it is challenging to integrate NGS data into daily workflows. The main challenge is to make sure that every patient with urologic cancer could have access to and take advantage of the information provided by NGS-based profiling [82].

5.5 Technological limitations in AI-based imaging and pathology

These are some of the major obstacles to the use of AI models in clinical urology: the necessity to harmonize the data acquired by disparate sources, issues related to ethical considerations, and the requirements of regulatory frameworks [83]. In order to be effectively deployed, especially in the diagnosis of bladder cancer, high-quality and large datasets of imaging modalities such as CT, MRI, and cystoscopy, which can be highly variable with regard to their acquisition protocols and resolution [46], and pathology data that can also be variable with regard to staining and microscopy methods are needed. In prostate cancer, it is especially difficult to develop uniform approaches to incorporating qualitative information in radiology, pathology, and clinical notes into quantitative measures to be used in decision-making. Because of the discrepancies in format, detail, and bias, combining these heterogeneous types of data into a single AI framework is challenging and remains a major barrier to producing robust and clinically useful predictions [84].

6. Advances in molecular targeted therapies

The progress in understanding the dysregulated pathways and the identification of key biomarkers in urologic cancers have been instrumental in the development of novel targeted therapies.

6.1 Tyrosine kinase inhibitors

Targeted therapies, Tyrosine kinase inhibitors (TKIs), are targeted cancer drugs that target tyrosine kinases, enzymes commonly mis-regulated in cancer. TKIs inhibiting VEGF and ET signaling (e.g., Pazopanib, Sorafenib, Cabozantinib) were initially designed to prevent angiogenesis in prostate cancer but demonstrated minor activity and significant toxicity in mCRPC trials [85, 86].

TKIs, such as Erdafitinib (FGFR1-4 inhibitor) and others (Pemigatinib, BGJ398, Rogaratinib) have come out promising in bladder cancer [87]. There are VEGFR-targeted TKIs (Nintedanib, Famininib, Sorafenib, Sunitinib), which are being studied, particularly in combination regimens [88].

TKIs targeting VEGFR and PDGFR (such as Sunitinib (first-line in clear cell RCC), Sorafenib, Axitinib, and Pazopanib) have developed outcomes in renal cell carcinoma (RCC), thereby indicating the angiogenesis caused by VHL inactivation [89, 90].

TKIs have had a minor role in testicular cancer, and trials of agents such as imatinib and sunitinib have not produced good results [91]. Their use in penile and urethral cancers is also being investigated, and trials are ongoing [92].

6.2 Inhibitors of major signaling proteins in driver pathways

A diverse range of inhibitors targeting major signaling proteins involved in cancer development and progression. These include inhibitors of DNA repair, cell growth, and epigenetic regulation.

This category includes several classes of agents in *prostate cancer*. The major inhibitors include those targeting the PI3K/mTOR/Akt pathway. The Akt inhibitor Ipatasertib has shown remarkable tumor cell growth inhibition in preclinical studies when combined with the androgen receptor antagonist Enzalutamide. PI3K/mTOR inhibitors like BEZ235 and CUDC-907 have demonstrated anti-tumor activity or the ability to re-sensitize resistant cancer cells to standard chemotherapeutic agents [85].

Research in *bladder cancer* is also actively exploring inhibitors of major signaling pathways. Dual inhibitors targeting both PI3K and mTOR have demonstrated the ability to suppress tumor cell growth in bladder cancer cell lines [88]. Src inhibitors showed disease stabilization with a proper therapeutic plan. Metformin is also being investigated for its anticancer properties. It targets metabolic pathways that are essential for tumor cell survival and proliferation [87].

Inhibitors of the mammalian target of rapamycin (mTOR) are approved for the treatment of *renal cell carcinoma*. This is particularly useful for patients who have progressed on VEGF-targeted therapy [89]. Inhibitors of the NF- κ B pathway are being studied in other diseases and may represent a future therapeutic approach for mRCC [90]. The *other urologic cancers* associated with von Hippel-Lindau (VHL) disease have HIF-2 alpha inhibitor Belzutifan as an approved therapy.

6.3 Synthetic lethal strategy (e.g., PARP inhibitors)

The concept of synthetic lethality refers to the combination of two non-lethal genetic or pharmacological perturbations resulting in cell death. This has been successfully exploited in urologic cancers and particularly with the development of PARP inhibitors.

PARP inhibitors have emerged as a significant therapeutic advancement for *prostate cancer*. This is an effective strategy for patients with metastatic castration-resistant disease and defects in DNA repair genes like *BRCA1/2* and other homologous recombination (HR) genes [85].

PARP inhibitors like Olaparib have shown limited activity in clinical trials for refractory TGCTs, with one trial reporting no complete or partial responses for *testicular carcinoma*. Another trial is investigating the combination of Veliparib with chemotherapy.

6.4 Immune checkpoint inhibitors

Immune checkpoint inhibitors (ICIs) represent a revolutionary class of targeted therapies that work by blocking proteins on immune cells. This includes blocking the immune cells like T-cells that normally help to keep these cells from attacking other cells in the body.

ICIs have not shown as much success in the case of *prostate cancer* as in some other urologic malignancies. This may be due to factors such as a low tumor mutational burden, low expression of the programmed death-ligand 1 (PD-L1), and a scarcity of T-cell infiltration within the tumor microenvironment [85].

The treatment of *bladder cancer* has been significantly impacted by the introduction of ICIs. These inhibitors block the interaction between PD-1 on T-cells and PD-L1 on tumor cells, which increases the anti-tumor immune response [88]. Several ICIs are approved for use in the first-line setting for cisplatin-ineligible patients with PD-L1-positive tumors and in second-line after failure of platinum-based chemotherapy [87].

ICIs targeting PD-1/PD-L1 and CTLA-4 have become a preferable choice for the treatment of metastatic *renal cell carcinoma* [93]. ICIs are being explored as a treatment option for *other urologic cancers* such as penile carcinoma. The early studies demonstrated promising results in some patients with advanced diseases. This was observed particularly for those tumors that exhibit PD-L1 expression and are associated with human papillomavirus (HPV) infection.

6.5 Androgen receptor targeted therapy

The traditional hormone therapy aims to reduce androgen levels or block their interaction with the AR in *prostate cancer*. Several latest AR-targeted drugs have demonstrated improved survival in patients with mCRPC. The combination of AR-targeted therapy with other molecularly targeted agents like PARP inhibitors has shown combined effects [85]. Other *urologic cancers* like bladder cancer and renal cell carcinoma are not very responsive to androgen receptor targeted therapy.

7. Challenges in molecular targeted therapies

Despite the remarkable progress in the development and application of molecular targeted therapies for urologic cancers, several significant challenges remain that can limit their effectiveness and widespread use.

7.1 Development of primary and acquired resistance

One of the most significant hurdles in the long-term success of molecular targeted therapies is the development of resistance. This resistance can be primary, in which the tumor is initially unresponsive to the therapy. It can also be acquired in which the tumor initially responds but then develops mechanisms to evade the drug's effects.

Cancer cells learn to adapt to the selective pressures imposed by targeted therapies. Mechanisms of resistance can include mutations in the drug target that prevent binding, activation of alternative signaling pathways that bypass the inhibited pathway, and changes in the tumor microenvironment that protect cancer cells from the drug.

7.2 Limited availability of predictive biomarkers

The future of personalized medicine in oncology relies on the ability to identify which patients are most likely to benefit from a specific targeted therapy. Multiple targeted agents used in urologic cancers and reliable predictive biomarkers are lacking or have inconsistent predictive power. Certain therapies have established biomarkers like *BRCA* mutations for PARP inhibitors in prostate cancer and *FGFR3/2* alterations for erdafitinib in bladder cancer. There are many other targeted therapies that lack efficient markers to guide treatment decisions [87].

7.3 Toxicity and adverse events with targeted agents

Molecular targeted therapies do not exist without their own set of toxicities and adverse events. These side effects can range from mild to severe and can significantly impact a patient's quality of life along with its ability to continue treatment. Examples of common toxicities associated with various TKIs, and other targeted agents used in urologic cancers include skin rashes, cardiovascular issues, fatigue, nausea, diarrhea, hyperphosphatemia, and ocular disorders [88]. Immune checkpoint inhibitors can also lead to immune-related adverse events.

8. Future directions and emerging technologies

The field of molecular targeted therapy for urologic cancers is characterized by rapid advancements and a continuous drive to overcome existing challenges along with developing more personalized treatments.

Combination therapies are expected to play an increasingly important role in the future of urologic cancer treatment [88]. The combination of targeted therapies with other treatment modalities, such as chemotherapy, radiotherapy, and other targeted agents (e.g., PARP inhibitors with AR inhibitors, TKIs with immunotherapies), is being investigated (**Figures 1** and **2** and **Table 1**).

9. Conclusion

Urologic malignancies comprise a heterogeneous collection of tumors that remain challenging to diagnose and treat despite the evidence from advancements in molecular biology, imaging, and directed treatments. Next-generation sequencing, liquid biopsies, and artificial intelligence have revolutionized diagnostics, facilitating the detection

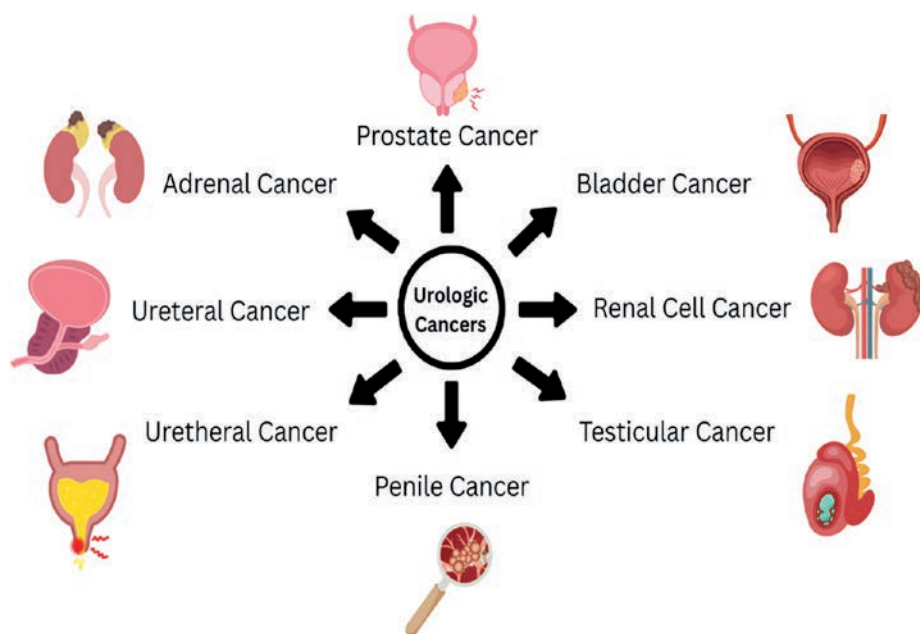


Figure 1.
Major types of urological cancers.

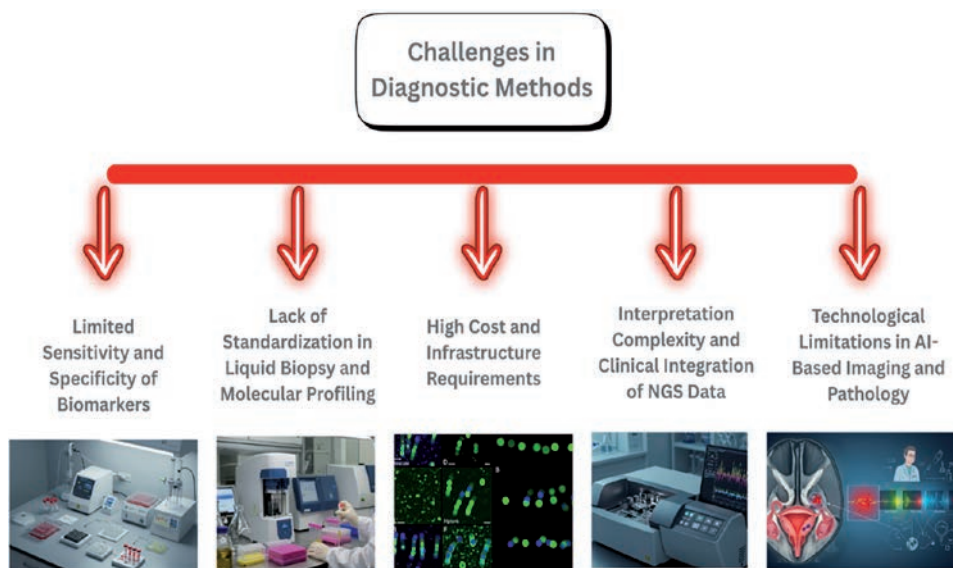


Figure 2.
Overview of challenges in diagnostic methods.

of malignancies at an earlier stage, enhancing the characterization of the tumors, and adopting more tailored treatment strategies. Yet, issues of biomarker specificity, financial cost, and standardization issues continue to prevail. Therapeutically, however, the advent of targeted therapies such as the use of tyrosine kinase inhibitors, immune checkpoint inhibitors, and synthetic lethal approaches such as PARP inhibitors

Cancer type	Drug name	Target	Indication	References
Prostate cancer	Olaparib	PARP	mCRPC with HR gene mutations after progression on hormonal agent	[85]
Prostate cancer	Rucaparib	PARP	mCRPC with BRCA1/2 mutations after hormonal agent and taxane therapy	[85]
Prostate cancer	Talazoparib	PARP	mCRPC with HRR alterations	[85]
Prostate cancer	Niraparib	PARP	mCRPC with BRCA1/2 mutations along with abiraterone and prednisone	[85]
Prostate cancer	Enzalutamide	AR	mCRPC, hormone-sensitive metastatic PCa, recurrence at high risk of metastasis	[85]
Prostate cancer	Abiraterone	AR	mCRPC, hormone-sensitive metastatic PCa, in combination with olaparib or niraparib	[85]
Prostate cancer	Darolutamide	AR	mCRPC, non-metastatic CRPC	[85]
Prostate cancer	Apalutamide	AR	mCRPC, non-metastatic CRPC, hormone-sensitive metastatic PCa	[85]
Bladder cancer	Erdafitinib	FGFR1-4	Advanced urothelial carcinoma with susceptible FGFR3/2 mutations after platinum-based chemotherapy	[88]
Bladder cancer	Atezolizumab	PD-L1	Metastatic urothelial carcinoma after platinum chemotherapy, cisplatin-ineligible PD-L, maintenance therapy	[87]
Bladder cancer	Nivolumab	PD-1	Advanced/metastatic urothelial carcinoma after platinum chemotherapy, high-risk muscle-invasive UC after radical surgery	[87]
Bladder cancer	Avelumab	PD-L1	Advanced/metastatic urothelial carcinoma after platinum chemotherapy, maintenance therapy after first platinum chemotherapy	[87]
RCC	Temsirolimus	mTOR	Poor-risk metastatic RCC	[90]
RCC	Everolimus	mTOR	Metastatic RCC after progression on VEGF-targeted therapy	[90]

Table 1.
Approved molecularly targeted therapies for urologic cancers (by cancer type and target).

improved outcomes in carefully selected urological malignancies. Still, resistance development, toxicity, and poor predictive biomarkers hinder optimal management. These issues will be overcome with ongoing commitment to translational research, solid clinical trials, and cross-disciplinary research. The future of urologic cancer treatment will come through the utilization of novel technologies such as nanomedicine, combination therapies, and precision drug delivery systems to overcome the existing constraints and provide genuinely individualized, effective treatment for all patients globally.

Acknowledgements

I express my deepest gratitude to my supervisor, Dr. Aneesa Sultan, for her guidance and support. AI assistance was utilized for sentence structuring and language

refinement in the abstract section of this chapter. This supported clarity and consistency in presentation.

Conflict of interest

The authors declare no conflict of interest.

Author details

Marium Jahan^{1*†}, Mohsin Ali^{1†}, Maryam Naeem¹, Muhammad Zakria¹, Muhammad Zuhair¹, Ayesha Rubab¹, Aneesa Sultan¹, Usman Tareen², Sabahat Qureshi¹ and Maryam Fida¹

1 Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

2 Department of Medical Technology, Islamabad Medical and Dental College, Pakistan

*Address all correspondence to: mjahan@bs.qau.edu.pk

† First two authors contributed equally.

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] Mohanty SK, Lobo A, Cheng L. The 2022 revision of the World Health Organization classification of tumors of the urinary system and male genital organs: Advances and challenges. *Human Pathology*. 2023;**136**:123-143
- [2] Zi H, Liu MY, Luo LS, Huang Q, Luo PC, Luan HH, et al. Global burden of benign prostatic hyperplasia, urinary tract infections, urolithiasis, bladder cancer, kidney cancer, and prostate cancer from 1990 to 2021. *Military Medical Research*. 2024;**11**(1):64
- [3] Dason S, Wong NC. Environmental and genetic factors contributing to bladder carcinogenesis. In: *Comprehensive Diagnostic Approach to Bladder Cancer: Molecular Imaging and Biomarkers*. Heidelberg, Germany; New York City, USA: Springer; 2021. pp. 13-44
- [4] Hoegger MJ, Strnad BS, Ballard DH, Siegel CL, Shetty AS, Weimholt RC, et al. Urinary bladder masses, rare subtypes, and masslike lesions: Radiologic-pathologic correlation. *Radiographics*. 2022;**43**(1):e220034
- [5] Kench JG, Amin MB, Berney DM, Compérat EM, Cree IA, Gill AJ, et al. WHO classification of tumours fifth edition: Evolving issues in the classification, diagnosis, and prognostication of prostate cancer. *Histopathology*. 2022;**81**(4):447-458
- [6] Chen J, Nelson WG, Sfanos K, Yegnasubramanian S, De Marzo AM. Molecular pathology of prostate cancer. In: *Molecular Surgical Pathology*. Cham: Springer International Publishing; 2023. pp. 377-399
- [7] Yu EM, Belay S, Li W, Aragon-Ching JB. Non-urothelial and urothelial variants of bladder cancer. *Cancer Treatment and Research Communications*. 2022;**33**:100661
- [8] Giudici N, Bonne F, Blarer J, Minoli M, Krentel F, Seiler R. Characteristics of upper urinary tract urothelial carcinoma in the context of bladder cancer: A narrative review. *Translational Andrology and Urology*. 2021;**10**(10):4036
- [9] Bukavina L, Bensalah K, Bray F, Carlo M, Challacombe B, Karam JA, et al. Epidemiology of renal cell carcinoma: 2022 update. *European Urology*. 2022;**82**(5):529-542
- [10] Rose TL, Kim WY. Renal cell carcinoma: A review. *JAMA*. 2024;**332**(12):1001-1010
- [11] Nauman M, Leslie S. *Nonseminomatous Testicular Tumors*. Treasure Island, FL, USA: StatPearls; 2023
- [12] Liu Q, Lian Q, Lv H, Zhang X, Zhou F. The diagnostic accuracy of miR-371a-3p for testicular germ cell tumors: A systematic review and meta-analysis. *Molecular Diagnosis & Therapy*. 2021;**25**:273-281
- [13] Thomas A, Necchi A, Muneer A, Tobias-Machado M, Tran ATH, Van Rompuy AS, et al. Penile cancer. *Nature Reviews Disease Primers*. 2021;**7**(1):11
- [14] Ahmed ME, Khalil MI, Kamel MH, Karnes RJ, Spiess PE. Progress on management of penile cancer in 2020. *Current Treatment Options in Oncology*. 2021;**22**:1-13
- [15] Lagarde-Lenon MS, Aron M. Female urethral carcinoma: A contemporary

review of the clinicopathologic features, with emphasis on the histoanatomic landmarks and potential staging issues. *Human Pathology*. 2022;**129**:71-80

[16] Lindner AK, Schachtner G, Tulchiner G, Thurnher M, Untergasser G, Obrist P, et al. Lynch syndrome: Its impact on urothelial carcinoma. *International Journal of Molecular Sciences*. 2021;**22**(2):531

[17] Mizdrak M, Tičinović Kurir T, Božić J. The role of biomarkers in adrenocortical carcinoma: A review of current evidence and future perspectives. *Biomedicine*. 2021;**9**(2):174

[18] Tamburello M. Carcinoma of the Human Adrenal Cortex: Identification and in Vitro and in Vivo Study of Molecular Alterations as Potential Therapeutic Targets. Brescia, Italy: University of Brescia; 2023

[19] Gómez-Palomo F, González JS, Gómez AB, Ardavín JP, Cerdá JR. Impact of aging on the incidence and mortality of urological cancers: 20-year projection in Spain. *Actas Urológicas Españolas (English Edition)*. 2022;**46**(5):268-274

[20] Wang L, Lu B, He M, Wang Y, Wang Z, Du L. Prostate cancer incidence and mortality: Global status and temporal trends in 89 countries from 2000 to 2019. *Frontiers in Public Health*. 2022;**10**:811044

[21] van Hoogstraten LM, Vrieling A, van der Heijden AG, Kogevinas M, Richters A, Kiemeny LA. Global trends in the epidemiology of bladder cancer: Challenges for public health and clinical practice. *Nature Reviews Clinical Oncology*. 2023;**20**(5):287-304

[22] Cirillo L, Innocenti S, Becherucci F. Global epidemiology of kidney cancer. *Nephrology Dialysis Transplantation*. 2024;**39**(6):920-928

[23] Giona S. The Epidemiology of Testicular Cancer. Brisbane, Australia: Exon Publications; 2022. pp. 107-116

[24] Giona S. The epidemiology of penile cancer. In: *Urologic Cancers* [Internet]. Exon Publications; 12 Sep 2022. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK585980/>

[25] Puglisi S, Calabrese A, Ferraù F, Violi MA, Laganà M, Grisanti S, et al. New findings on presentation and outcome of patients with adrenocortical cancer: Results from a national cohort study. *The Journal of Clinical Endocrinology & Metabolism*. 2023;**108**(10):2517-2525

[26] Hansen M, Hamieh NM, Markt SC, Vaselkiv JB, Pernar CH, Gonzalez-Feliciano AG, et al. Racial disparities in prostate cancer: Evaluation of diet, lifestyle, family history, and screening patterns. *Cancer Epidemiology, Biomarkers & Prevention*. 2022;**31**(5):982-990

[27] Lobo N, Afferi L, Moschini M, Mostafid H, Porten S, Psutka SP, et al. Epidemiology, screening, and prevention of bladder cancer. *European Urology Oncology*. 2022;**5**(6):628-639

[28] Campi R, Rebez G, Klatte T, Roussel E, Ouizad I, Ingels A, et al. Effect of smoking, hypertension and lifestyle factors on kidney cancer—Perspectives for prevention and screening programmes. *Nature Reviews Urology*. 2023;**20**(11):669-681

[29] Yazici S, Del Biondo D, Napodano G, Grillo M, Calace FP, Prezioso D, et al. Risk factors for testicular cancer: Environment, genes and infections—Is it all? *Medicina*. 2023;**59**(4):724

[30] Mannam G, Miller JW, Johnson JS, Gullapalli K, Fazili A, Spiess PE, et al.

HPV and penile cancer: Epidemiology, risk factors, and clinical insights. *Pathogens*. 2024;**13**(9):809

[31] Challacombe B, Bultitude MF. The kidneys, urinary tract and prostate. In: *Browse's Introduction to the Symptoms & Signs of Surgical Disease*. Boca Raton, FL, USA: CRC Press; 2021. pp. 573-587

[32] Scatolini M, Grisanti S, Tomaiuolo P, Grosso E, Basile V, Cosentini D, et al. Germline NGS targeted analysis in adult patients with sporadic adrenocortical carcinoma. *European Journal of Cancer*. 2024;**205**:114088

[33] Haffner MC, Zwart W, Roudier MP, True LD, Nelson WG, Epstein JI, et al. Genomic and phenotypic heterogeneity in prostate cancer. *Nature Reviews Urology*. 2021;**18**(2):79-92

[34] Singh R, Mills IG. The interplay between prostate cancer genomics, metabolism, and the epigenome: Perspectives and future prospects. *Frontiers in Oncology*. 2021;**11**:704353

[35] Iyyanki T, Zhang B, Wang Q, Hou Y, Jin Q, Xu J, et al. Subtype-associated epigenomic landscape and 3D genome structure in bladder cancer. *Genome Biology*. 2021;**22**:1-20

[36] Fukagawa A, Hama N, Totoki Y, Nakamura H, Arai Y, Saito-Adachi M, et al. Genomic and epigenomic integrative subtypes of renal cell carcinoma in a Japanese cohort. *Nature Communications*. 2023;**14**(1):8383

[37] Nuñez-Corona D, Contreras-Sanzón E, Puente-Rivera J, Arreola R, Camacho-Nuez M, Cruz Santiago J, et al. Epigenetic factors and ncRNAs in testicular cancer. *International Journal of Molecular Sciences*. 2023;**24**(15):12194

[38] Necchi A, Spiess PE, de Padua TC, Li R, Grivas P, Huang RS, et al. Genomic profiles and clinical outcomes of penile squamous cell carcinoma with elevated tumor mutational burden. *JAMA Network Open*. 2023;**6**(12):e2348002-e2348002

[39] Obinata D, Takayama K, Inoue S, Takahashi S. Exploring androgen receptor signaling pathway in prostate cancer: A path to new discoveries. *International Journal of Urology*. 2024;**31**(6):590-597

[40] Rezaei S, Nikpanjeh N, Rezaee A, Gholami S, Hashemipour R, Biavarz N, et al. PI3K/Akt signaling in urological cancers: Tumorigenesis function, therapeutic potential, and therapy response regulation. *European Journal of Pharmacology*. 2023;**955**:175909

[41] Onorato A, Guida E, Colopi A, Dolci S, Grimaldi P. RAS/mitogen-activated protein kinase signaling pathway in testicular germ cell tumors. *Life*. 2024;**14**(3):327

[42] Iacovelli R, Arduini D, Ciccarese C, Pierconti F, Strusi A, Piro G, et al. Targeting hypoxia-inducible factor pathways in sporadic and Von Hippel-Lindau syndrome-related kidney cancers. *Critical Reviews in Oncology/Hematology*. 2022;**176**:103750

[43] Yu C, Zheng B, Zhang L, Zhang A, Jia Z, Ding G. Wnt/ β -catenin signaling and congenital abnormalities of kidney and urinary tract. *Kidney Diseases*. 2024;**10**(6):588-599

[44] Manzano RG, Catalan-Latorre A, Brugarolas A. RB1 and TP53 co-mutations correlate strongly with genomic biomarkers of response to immunity checkpoint inhibitors in urothelial bladder cancer. *BMC Cancer*. 2021;**21**:1-23

- [45] Ding L, Dong HY, Zhou TR, Wang YH, Yan T, Li JC, et al. PD-1/PD-L1 inhibitors-based treatment for advanced renal cell carcinoma: Mechanisms affecting efficacy and combination therapies. *Cancer Medicine*. 2021;**10**(18):6384-6401
- [46] Eskandar K. Artificial intelligence in urology: Revolutionizing diagnostics and treatment planning. *Arab Journal of Urology*. 2024:1-7. DOI: 10.1080/20905998.2024.2443334 [Online first]
- [47] Narain TA, Sooriakumaran P. Beyond prostate specific antigen: New prostate cancer screening options. *The World Journal of Men's Health*. 2022;**40**(1):66
- [48] Flores Monar GV, Reynolds T, Gordon M, Moon D, Moon C. Molecular markers for bladder cancer screening: An insight into bladder cancer and FDA-approved biomarkers. *International Journal of Molecular Sciences*. 2023;**24**(18):14374
- [49] Karmakova TA, Sergeeva NS, Kanukoev KY, Alekseev BY, Kaprin AD. Kidney injury molecule 1 (KIM-1): A multifunctional glycoprotein and biological marker. *Современные технологии в медицине*. 2021;**13**(3 (eng)):64-78
- [50] Sarkis J, Kourie HR, Alkassis M, Samaha R, Sarkis P. Novel biomarkers in testicular germ cell tumors: Updates of 2020. *Biomarkers in Medicine*. 2021;**15**(2):83-86
- [51] Bologna E, Ditunno F, Licari LC, Franco A, Manfredi C, Mossack S, et al. Tissue-based genomic testing in prostate cancer: 10-year analysis of national trends on the use of Prolaris, Decipher, ProMark, and Oncotype DX. *Clinics and Practice*. 2024;**14**(2):508-520
- [52] Chacko N, Ankri R. Non-invasive early-stage cancer detection: Current methods and future perspectives. *Clinical and Experimental Medicine*. 2024;**25**(1):17
- [53] Huang Q, Mitsiades I, Dowst H, Zarrin-Khameh N, Noor AB, Castro P, et al. Incidental detection of FGFR3 fusion via liquid biopsy leading to earlier diagnosis of urothelial carcinoma. *npj Precision Oncology*. 2023;**7**(1):123
- [54] Enikeev D, Morozov A, Babaevskaya D, Bazarkin A, Malavaud B. A systematic review of circulating tumor cells clinical application in prostate cancer diagnosis. *Cancers*. 2022;**14**(15):3802
- [55] Li K, Wang Q, Tang X, Akakuru OU, Li R, Wang Y, et al. Advances in prostate cancer biomarkers and probes. *Cyborg and Bionic Systems*. 2024;**5**:0129
- [56] Alahdal M, Perera RA, Moschovas MC, Patel V, Perera RJ. Current advances of liquid biopsies in prostate cancer: Molecular biomarkers. *Molecular Therapy-Oncolytics*. 2023;**30**:27-38
- [57] Dwivedi DK, Jagannathan NR. Emerging MR methods for improved diagnosis of prostate cancer by multiparametric MRI. *Magnetic Resonance Materials in Physics, Biology and Medicine*. 2022;**35**(4):587-608
- [58] Huang S, Ong S, McKenzie D, Mirabelli A, Chen DC, Chengodu T, et al. Comparison of 18F-based PSMA radiotracers with [68Ga] Ga-PSMA-11 in PET/CT imaging of prostate cancer—A systematic review and meta-analysis. *Prostate Cancer and Prostatic Diseases*. 2024;**27**(4):654-664
- [59] Urso L, Bauckneht M, Albano D, Chondrogiannis S, Grassetto G, Lanfranchi F, et al. The evolution of PET imaging in renal, bladder, upper urinary

tract urothelial, testicular and penile carcinoma–Today’s impact, tomorrow’s potential. *Expert Review of Medical Devices*. 2024;**21**(1-2):55-72

[60] Roussel E, Campi R, Amparore D, Bertolo R, Carbonara U, Erdem S, et al. Expanding the role of ultrasound for the characterization of renal masses. *Journal of Clinical Medicine*. 2022;**11**(4):1112

[61] Ji A, Cheng Z. Imaging of biological processes using positron emission tomography and single-photon emission computed tomography. In: *Transpathology*. Amsterdam, Netherlands: Elsevier; 2024. pp. 121-131

[62] Ascione CM, Napolitano F, Esposito D, Servetto A, Belli S, Santaniello A, et al. Role of FGFR3 in bladder cancer: Treatment landscape and future challenges. *Cancer Treatment Reviews*. 2023;**115**:102530

[63] Pezzicoli G, Ciciriello F, Musci V, Salonne F, Ragno A, Rizzo M. Genomic profiling and molecular characterization of clear cell renal cell carcinoma. *Current Oncology*. 2023;**30**(10):9276-9290

[64] Grypari IM, Tzelepi V, Gyftopoulos K. DNA damage repair pathways in prostate cancer: A narrative review of molecular mechanisms, emerging biomarkers and therapeutic targets in precision oncology. *International Journal of Molecular Sciences*. 2023;**24**(14):11418

[65] Chen S. *The Transcriptomic Heterogeneity in Prostate Cancer*. Canada: University of Toronto; 2021

[66] Feretzakis G, Juliebø-Jones P, Tsaturyan A, Sener TE, Verykios VS, Karapiperis D, et al. Emerging trends in AI and radiomics for bladder, kidney, and prostate cancer: A critical review. *Cancers*. 2024;**16**(4):810

[67] Shafi S, Parwani AV. Artificial intelligence in diagnostic pathology. *Diagnostic Pathology*. 2023;**18**(1):109

[68] Salem H, Soria D, Lund JN, Awwad A. A systematic review of the applications of Expert Systems (ES) and machine learning (ML) in clinical urology. *BMC Medical Informatics and Decision Making*. 2021;**21**:1-36

[69] Fawaz A, Ferraresi A, Isidoro C. Systems biology in cancer diagnosis integrating omics technologies and artificial intelligence to support physician decision making. *Journal of Personalized Medicine*. 2023;**13**(11):1590

[70] Urquidi V, Rosser CJ, Goodison S. Molecular diagnostic trends in urological cancer: Biomarkers for non-invasive diagnosis. *Current Medicinal Chemistry*. 2012;**19**(22):3653-3663

[71] Martignano F, Rossi L, Maugeri A, Gallà V, Conteduca V, De Giorgi U, et al. Urinary RNA-based biomarkers for prostate cancer detection. *Clinica Chimica Acta*. 2017;**473**:96-105

[72] Oshi M, Murthy V, Takahashi H, Huyser M, Okano M, Tokumaru Y, et al. Urine as a source of liquid biopsy for cancer. *Cancers*. 2021;**13**(11):2652

[73] Bulai C, Geavlete P, Ene CV, Bulai I, Popescu RI, Mares C, et al. Detection of urinary molecular marker test in urothelial cell carcinoma: A review of methods and accuracy. *Diagnostics*. 2022;**12**(11):2696

[74] Huang J, Huang D, Ruan X, Zhan Y, Chun STT, Ng ATL, et al. Clinical translational research of liquid biopsy applications in prostate cancer and other urological cancers. *Cambridge Prisms: Precision Medicine*. 2023;**1**:e33

- [75] Rodríguez J, Avila J, Rolfo C, Ruíz-Patiño A, Russo A, Ricaurte L, et al. When tissue is an issue the liquid biopsy is nonissue: A review. *Oncology and Therapy*. 2021;**9**:89-110
- [76] Godlewski D, Bartusik-Aebisher D, Czech S, Szpara J, Aebisher D. Bladder cancer biomarkers. *Exploration of Targeted Anti-tumor Therapy*. 2025;**6**:1002301
- [77] Muhammad U, Ahmad U, Ibrahim B, Ahmad AA, Liman HU. A systematic review on diagnostic and prognostic biomarkers for bladder cancer. *medRxiv*. 2024. DOI: 10.1101/2024.06.02.24308331 [Preprint]
- [78] Lee HH, Kim SH. Review of non-invasive urinary biomarkers in bladder cancer. *Translational Cancer Research*. 2020;**9**(10):6554
- [79] Wan X, Wang D, Zhang X, Xu M, Huang Y, Qin W, et al. Unleashing the power of urine-based biomarkers in diagnosis, prognosis and monitoring of bladder cancer. *International Journal of Oncology*. 2025;**66**(3):1-22
- [80] Schmid S, Jochum W, Padberg B, Demmer I, Mertz KD, Joerger M, et al. How to read a next-generation sequencing report—What oncologists need to know. *ESMO Open*. 2022;**7**(5):100570
- [81] Karakoyun HK, Sayar C, Yazarbaş K. Challenges in clinical interpretation of next-generation sequencing data: Advantages and Pitfalls. *Results in Engineering*. 2023;**20**:101421
- [82] Zalis M, Viana Veloso GG, Aguiar PN Jr, Gimenes N, Reis MX, Matsas S, et al. Next-generation sequencing impact on cancer care: Applications, challenges, and future directions. *Frontiers in Genetics*. 2024;**15**:1420190
- [83] Singh S, Shukla RM. Artificial intelligence: The latest advances in the diagnosis of bladder cancer. *Journal of Urologic Oncology*. 2024;**22**(3):268-280
- [84] Pai RK, Van Booven DJ, Parmar M, Lokeshwar SD, Shah K, Ramasamy R, et al. A review of current advancements and limitations of artificial intelligence in genitourinary cancers. *American Journal of Clinical and Experimental Urology*. 2020;**8**(5):152
- [85] Sorrentino C, Di Carlo E. Molecular targeted therapies in metastatic prostate cancer: Recent advances and future challenges. *Cancers*. 2023;**15**(11):2885
- [86] Gao P, Li T, Zhang K, Luo G. Recent advances in the molecular targeted drugs for prostate cancer. *International Urology and Nephrology*. 2023;**55**(4):777-789
- [87] Peng M, Chu X, Peng Y, Li D, Zhang Z, Wang W, et al. Targeted therapies in bladder cancer: Signaling pathways, applications, and challenges. *MedComm*. 2023;**4**(6):e455
- [88] Bell SD, Quinn AE, Spitzer TD, Voss BB, Wakefield MR, Fang Y. Emerging molecular therapies in the treatment of bladder cancer. *Exploration of Targeted Anti-tumor Therapy*. 2024;**5**(5):1135
- [89] Yuen JS. Molecular targeted therapy in advanced renal cell carcinoma: A review of its recent past and a glimpse into the near future. *Indian Journal of Urology*. 2009;**25**(4):427-436
- [90] Cho IC, Chung J. Current status of targeted therapy for advanced renal cell carcinoma. *Korean Journal of Urology*. 2012;**53**(4):217
- [91] Mele T, Reid A, Huddart R. Recent advances in testicular germ cell tumours. *Faculty Reviews*. 2021;**10**:67

[92] Benjamin DJ, Hsu RC. Treatment approaches in advanced penile cancer: Targeted therapies and immunotherapy. *Frontiers in Oncology*. 2025;**14**:1457006

[93] Li X, Xiong W, Xiong Z, Zhang X. Molecular mechanisms of renal cell carcinoma metastasis and potential targets for therapy. *Frontiers in Cell and Developmental Biology*. 2025;**13**:1521151

Section 2

Prostate Cancer: From Molecular Subtypes to Overcoming Resistance

Neuroendocrine Prostate Cancer: An Overlooked Identity?

Nastasiia Artamonova and Isabel Heidegger

Abstract

Neuroendocrine prostate cancer (NEPC) is a rare and aggressive subtype of prostate cancer, presenting significant diagnostic and therapeutic challenges. NEPC arises in two distinct forms: de novo NEPC, affecting less than 2% of cases, and treatment-emergent NEPC (t-NEPC), which develops in up to 30% of patients with castration-resistant prostate cancer (CRPC). This aggressive variant is characterized by androgen receptor (AR)-independent growth, resistance to conventional hormone therapies, rapid progression, and frequent visceral metastases. Diagnosis of NEPC remains challenging due to the absence of prostate-specific antigen (PSA) elevation, limitations in tissue sampling, and reliance on advanced imaging techniques like fluorodeoxyglucose-positron emission tomography (FDG-PET). Current treatments rely on platinum-based chemotherapy, but outcomes remain poor, with median survival under 17 months. Emerging therapies focus on molecular alterations, including phosphatase and tensin homolog (PTEN) loss, breast cancer gene 1 (BRCA1), breast cancer gene 2 (BRCA2) mutations, and delta-like ligand 3 (DLL3) expression. Despite advances in understanding NEPC biology, effective treatments remain limited, underscoring the urgent need for novel therapeutic strategies to improve patients' survival and quality of life.

Keywords: neuroendocrine prostate cancer, neuroendocrine transdifferentiation, small cell prostate cancer, treatment-emergent neuroendocrine prostate cancer, tumor microenvironment

1. Introduction

Generally, neuroendocrine prostate cancer (NEPC) can manifest in two forms: The first one, known as de novo NEPC, is exceptionally rare, affecting less than 2% of all prostate cancer patients [1]. The second, and more prevalent, form develops through changes in androgen receptor (AR) signaling or the adoption of neuroendocrine characteristics. This transition typically occurs during treatment with AR inhibitors or chemotherapy drugs in the taxane family [2, 3]. This transformation results in what is known as treatment-emergent neuroendocrine prostate cancer (t-NEPC), affecting up to 30% of patients with castration-resistant prostate cancer (CRPC) [4].

The shift to NEPC poses significant challenges in both treatment and diagnosis. As the tumor becomes independent of androgen signaling, traditional therapies such

as androgen deprivation therapy (ADT) and AR pathway inhibitors (ARPI) become ineffective. Currently, a significant clinical challenge remains in both identifying and treating the de novo as well as t-NEPC. Particularly challenging is the management of small cell neuroendocrine carcinoma (SCNC), an aggressive NEPC subtype, where treatment options currently rely primarily on platinum-based chemotherapy, highlighting the urgent need for novel treatment strategies [5].

2. Histopathological features

Neuroendocrine cells are rare within the prostate, comprising approximately 1% of the epithelial population, and are scattered among more abundant luminal and basal cells [6, 7]. Their scarcity in normal prostate tissue and most prostate tumors makes them difficult to detect using standard hematoxylin and eosin (H&E) staining. A more effective method for identifying neuroendocrine cells in the prostate epithelium is immunohistochemistry (IHC), which uses markers such as chromogranin A (CgA), synaptophysin (SYP), neuron-specific enolase (NSE), neural cell adhesion molecule (NCAM or CD56), forkhead-box A2 (FOXA2), thyroid transcription factor-1 (TTF-1), p53, and CXC chemokine receptor 2 (CXCR2) [6–10]. Neuroendocrine cells are negative for AR and prostate-specific antigen (PSA) and also lack Ki67 expression, reflecting their non-proliferative, post-mitotic nature [6, 8, 10].

The World Health Organization's 2022 Classification provides a comprehensive framework for categorizing NEPC based on distinct morphological patterns. The classification system recognizes both well-differentiated and poorly differentiated forms. Well-differentiated variants include prostate adenocarcinoma with neuroendocrine differentiation, adenocarcinoma showing Paneth cell neuroendocrine features, and carcinoid tumors. The poorly differentiated category encompasses small cell carcinoma, large cell neuroendocrine carcinoma (LCNC), and mixed neuroendocrine carcinoma-acinar adenocarcinoma [11]. It is important to note that while NEPC typically exhibits specific neuroendocrine markers through IHC, the presence of these markers is not mandatory for diagnosis [12].

According to the current literature, SCNC is the most common subtype of NEPC. Histologically, SCNC is characterized by features such as a high nuclear-to-cytoplasmic ratio, scant cytoplasm, hyperchromatic nuclei, nuclear molding, frequent mitoses, and necrosis [12, 13]. Unlike benign neuroendocrine cells or those in adenocarcinoma, SCNC tumor cells are highly proliferative and exhibit elevated levels of Ki67 expression [12, 13]. Pure SCNC is rare; it is more commonly observed as a mixed histology with adenocarcinoma components. In these mixed cases, the SCNC component varies in extent, while the adenocarcinoma typically has a Gleason grade of 8 or higher [14].

Other NEPC subtypes include carcinoid tumors and LCNC. Carcinoid tumors are extremely rare and consist of well-differentiated nests of uniform neuroendocrine cells that are positive for neuroendocrine markers but are not associated with adenocarcinoma. Their clinical relevance as de novo prostate cancer remains unclear due to limited case reports [15, 16]. LCNC can be distinguished from SCNC by larger cell size, abundant cytoplasm, prominent nucleoli, and peripheral palisading [14, 17, 18]. Historically thought to arise primarily after hormone therapy, recent evidence suggests that nearly half of LCNC cases may occur de novo [19, 20]. Regardless of origin, LCNC is associated with rapid progression, with a median

survival ranging from 21.5 months for de novo and 5 months in patients with metastatic disease [21].

The prognosis of other NEPC subtypes varies significantly – well-differentiated forms like Paneth cell and carcinoid tumors generally have better outcomes, while poorly differentiated variants such as SCNC are more aggressive [22].

3. Diagnosis of NEPC

The diagnosis of NEPC remains challenging despite a growing understanding of its clinical and pathological characteristics. Several factors contribute to these difficulties. Firstly, the primary diagnostic method relies on tissue biopsy, which presents limitations due to its invasive nature, and the frequent occurrence of bone metastases makes tissue sampling more invasive compared to biopsies of other organs. Secondly, the absence of standardized guidelines regarding timing and anatomical sites for biopsy further complicates the diagnostic process. Unlike prostate adenocarcinoma, NEPC does not produce PSA, making PSA-based screening methods ineffective for its detection [6]. The disease also frequently lacks expression of prostate-specific membrane antigen (PSMA), which compromises the accuracy of PSMA-based imaging techniques. To overcome these limitations, alternative imaging methods such as fluorodeoxyglucose-positron emission tomography (FDG-PET) may be effective for detecting NEPC tumors due to their high metabolic activity [23]. Imaging studies have shown that up to one-third of patients (91 out of 291 men) screened for the TheraP trial exhibited PSMA-low or PSMA/FDG discordant lesions. While these imaging features have been linked to poor outcomes, the exact relationship between PSMA/FDG imaging characteristics and tumor pathology remains unclear [24].

Patients with NEPC often present with distinct clinical features that differentiate them from typical prostate adenocarcinoma cases. Disease progression in NEPC frequently occurs without a corresponding rise in serum PSA levels, although this is not universally observed [21, 25–27]. Instead, patients may show elevated serum levels of neuroendocrine markers such as chromogranin A, neuron-specific enolase (NSE), or carcinoembryonic antigen (CEA). Additionally, the metastatic pattern in NEPC also differs, with patients more likely to develop metastases in unusual locations, such as visceral organs and the brain [27].

4. De novo NEPC

The molecular mechanisms underlying de novo NEPC remain poorly understood, primarily due to the limited availability of tumor samples and the rapid disease progression, characteristic of this aggressive subtype. Another challenge arises from the fact that most genomic studies on NEPC do not clearly distinguish between de novo and treatment-emergent cases, leading to ambiguity in subtype-specific molecular profiles. To the best of our knowledge, a review of the current literature identifies only four studies explicitly reporting de novo NEPC cases, collectively describing 51 patients [28]. This scarcity of well-characterized cases hinders comprehensive molecular characterization and underscores the need for dedicated studies focusing on de novo NEPC biology.

A retrospective analysis of 18 de novo SCNC cases revealed genetic alterations similar to those found in treatment-related SCNC, including biallelic loss of TP53

and retinoblastoma protein 1 (RB1), two tumor suppressor genes critical for regulating cell cycle progression and apoptosis [29]. The loss of these genes has profound implications for tumor behavior, as RB1 regulates the cell cycle while TP53 controls apoptotic pathways. Their combined inactivation drives the aggressive phenotype of SCNC, characterized by rapid proliferation and resistance to cell death. In vitro studies using androgen-resistant prostate cancer cells in mouse models have demonstrated that RB1 deletion promotes lineage plasticity [30]. This mechanism allows cancer cells to adapt by acquiring characteristics of different cell lineages that are no longer dependent on the original drug target. This transformation is mediated through the upregulation of key epigenetic and transcriptional reprogramming factors, including SRY-box transcription factor 2 (SOX2) and the histone methyltransferase enhancer of zeste homolog 2 (EZH2), both of which facilitate the transition to a neuroendocrine state [31]. In a subset of patients, complete loss of key DNA repair genes, including breast cancer gene 1 (BRCA1), breast cancer gene 2 (BRCA2), ataxia-telangiectasia mutated (ATM), and MutS homolog 2 (MSH2) and MutS homolog 6 (MSH6), was observed, potentially explaining the high genomic instability observed in this cancer subtype [29]. Notably, two patients with pure de novo SCNC harbored ETS gene rearrangements associated with androgen-regulated promoters. This discovery suggests that even de novo SCNC, which is typically considered androgen-independent, may originate from cells that were once responsive to androgens [29].

Additionally, one of the studies revealed a phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) mutation in one case of SCNC [32]. While PIK3CA mutations are known to accelerate tumorigenesis and progression to CRPC, this specific alteration has not been previously documented in prostatic SCNC [33].

5. t-NEPC

There are two main and partly conflicting models explaining the origin of t-NEPC. The first model proposes that t-NEPC develops through clonal expansion of pre-existing basal or neuroendocrine cells naturally present in small numbers within the healthy prostate. These cells might have a growth advantage in the absence of androgen signaling [8, 34, 35]. This hypothesis is supported by lineage tracing in transgenic mouse models [34]. The second model suggests that t-NEPC arises through the transdifferentiation of adenocarcinoma cells. This is supported by evidence showing identical ERG gene rearrangements and shared AR amplifications in both small cell carcinoma and adenocarcinoma regions from the same patient [36, 37]. In this model, the loss of AR expression in t-NEPC occurs through epigenetic or post-transcriptional modifications rather than direct genomic loss [38]. Notably, studies have shown that re-exposure to androgens can reverse neuroendocrine differentiation in certain models, further supporting this transdifferentiation hypothesis [39]. Both models could potentially contribute to the development of t-NEPC in different contexts as well as in different subsets of patients.

Research has identified significant overexpression and amplification of Aurora kinase A (AURKA) and N-myc in NEPC, demonstrating their role in driving neuroendocrine differentiation in prostate cells [40]. AURKA plays an important role in stabilizing N-myc, as observed in other neuroendocrine tumors like neuroblastoma [41]. In NEPC, N-myc induces neuroendocrine differentiation through suppression of AR signaling and enhancement of polycomb repressive complex 2 (PRC2) target gene repression [42]. Specifically, p53 mutation leads to increased expression

of microRNA-25, which downregulates F-Box and WD repeat domain containing 7 (FBXW7), an E3 ubiquitin ligase responsible for AURKA degradation [43]. This results in elevated AURKA levels, promoting rapid proliferation and aggressive behavior in NEPC. Recent studies have identified that neurokinin-1 receptor (NK1R) expression is elevated in prostate cancer, particularly in metastatic cases and t-NEPC, suggesting its role in the progression from adenocarcinoma to NEPC. Importantly, AR inhibition leads to increased NK1R expression, which in turn activates the protein kinase C alpha (PKC α)-AURKA/N-myc signaling pathway, creating a feed-forward loop that promotes neuroendocrine differentiation. Notably, targeting NK1R has been shown to prevent neuroendocrine transdifferentiation and reduce tumor growth both in vitro and in vivo, suggesting its potential as a therapeutic target [44].

t-NEPC development is closely linked to the deregulation of signaling pathways that control lineage plasticity, stem-like behavior, and epithelial-to-mesenchymal transition (EMT). Research has shown that basal markers like trophoblast cell surface antigen 2 (TROP2) and EMT inducers such as SNAIL have been shown to independently induce neuroendocrine phenotypes in prostate cancer models [45]. The loss of AR signaling drives the tumor to rely on alternative metabolic and survival pathways. A notable example is the increased activity of sterol regulatory element-binding proteins (SREBPs), which enhance fatty acid and cholesterol production, enabling tumor growth even without androgen signaling [46, 47]. In addition, cAMP response element-binding protein (CREB) plays a key role in SREBP1 activation through mammalian target of rapamycin (mTOR) signaling appears to support cell viability in t-NEPC, indicating a potentially important CREB-SREBP axis in managing tumor growth [47].

In vitro and in vivo studies have shown that tumors can develop resistance to enzalutamide, a widely used ARPI, by transforming AR-dependent luminal epithelial cells to AR-independent basal-like cells. This transition to a neuroendocrine phenotype in t-NEPC is often associated with the loss of p53 function, in addition to pre-existing RB1 loss and phosphatase and tensin homologue (PTEN) mutations [3, 30]. The shift toward a neuroendocrine state is primarily driven by increased expression of SOX2, a key transcription factor involved in cellular reprogramming [31]. These results were further validated in a mouse model that has demonstrated RB1 loss, which promotes lineage plasticity and metastasis in prostate adenocarcinomas initially triggered by PTEN mutations [30]. The additional inactivation of Trp53 (the mouse homolog of p53) was found to induce resistance to antiandrogen therapies. Moreover, gene expression analyses in these models revealed upregulation of epigenetic reprogramming factors, particularly EZH2 and SOX2 [30]. Importantly, EZH2 inhibitors were shown to have clinical relevance as they have the ability to reinstate androgen receptor expression and restore sensitivity to antiandrogen treatments [42, 48]. Another factor that was found to contribute to lineage plasticity in t-NEPC is mucin 1 (MUC1-C), a transmembrane protein that was evolutionarily designed to maintain tissue homeostasis [49]. Through its chronic activation through inflammation, MUC1-C promotes lineage plasticity and carcinogenesis [50]. In NEPC cell models, MUC1-C has been shown to suppress AR signaling, directly interact with Myc, and activate neural transcription factors, including brain-specific homeobox/POU domain protein (BRN)2 and achaete-scute family BHLH transcription factor 1 (ASCL1), which are crucial for the neuroendocrine phenotype. Additionally, MUC1-C induces neurogenic locus notch homologue protein 1 (NOTCH1), promoting cancer stem cell characteristics [51]. The molecular impact of MUC1-C extends to epigenetic

regulation through its interaction with switch/sucrose non-fermentable complex (SWI/SNF) embryonic stem Brg/Brahma-associated factor (esBAF) and polybromomethyl BAF (PBAF) chromatin remodeling complexes, leading to widespread changes in chromatin architecture [52]. These alterations incorporate cancer stem cell properties, including the regulation of oxidative stress within the cell and enhancement of its self-renewal capacity [53]. This dependency on MUC1-C appears to be shared across various types of NEPC, including small cell lung cancer (SCLC) and Merkel cell carcinoma, suggesting its potential as a therapeutic target for aggressive neuroendocrine malignancies [54, 55].

Chromatin landscape of t-NEPC was shown to exhibit similarities to SCLC with distinct subtypes based on the expression of the neuronal transcription factors ASCL1 and neurogenic differentiation factor 1 (NEUROD1). Single-cell sequencing of human clinical samples revealed a more intricate tumor structure, where these subtypes coexist as separate subpopulations within individual tumors [56]. Recent single-cell analyses have revealed more complex subpopulations, including cells co-expressing achaete-scute family BHLH transcription factor 2 (ASCL2) and POU class 2 homeobox 3 (POU2F3). In prostate cancer, ASCL2+ cells are frequently observed in CRPC, suggesting a role in transdifferentiation. The interplay between ASCL1 and ASCL2 appears crucial, with ASCL1 promoting ASCL2 expression while ASCL2 suppresses ASCL1 [56–58].

6. Tumor microenvironment in NEPC

Cancer-associated fibroblasts (CAFs) are a key component of the tumor microenvironment (TME) and play a significant role in the progression of NEPC. Angiogenesis, along with tumor progression, is strongly influenced by CAFs through their secretion of cytokines and other signaling molecules [59]. ADT has been shown to alter prostate fibroblast activity; these fibroblasts show increased DNA methyltransferase activity, leading to RASAL3 suppression and enhanced glutamine secretion [60, 61]. This metabolic shift promotes tumor cell proliferation, activates mTOR signaling cascades, and upregulates neuroendocrine markers, particularly enolase 2 (ENO2) [60]. Additionally, the inflammatory milieu within the TME significantly influences neuroendocrine differentiation through cytokine-mediated signaling networks. Tumor-associated macrophages and CAFs secrete interleukin (IL)-6, which triggers the expression of neuroendocrine markers through STAT3 and mitogen-activated protein kinase (MAPK) pathways while promoting angiogenesis via phosphoinositide 3-kinases/protein kinase B (PI3K/Akt) signaling [62, 63]. Furthermore, IL-8, typically repressed by forkhead box A1 (FOXA1), becomes elevated in NEPC and supports tumor survival through similar signaling cascades [64].

NEPC cells actively drive tumor progression through autocrine and paracrine signaling. They secrete various neuropeptides, including survivin, gastrin-releasing peptide, neurotensin, and serotonin, which promote tumor growth, survival, and angiogenesis [65]. These factors signal through multiple receptors, including epidermal growth factor receptor (EGFR) and insulin-like growth factor 1 receptor (IGF1-R), activating various downstream pathways [66]. The secretion process is facilitated by calcium-dependent mechanisms and specific ion channels, with evidence suggesting that AR suppression can alter neurotransmitter release patterns

[67]. NEPC cells also communicate through extracellular vesicles, which transport crucial regulatory molecules, including transcription factors BRN2 and BRN4 [68, 69]. These factors contribute to the induction of neuroendocrine features and androgen independence in recipient cells. BRN2, which is suppressed by AR signaling, regulates the expression of SOX2, a key stem cell transcription factor [70].

Hypoxia plays a crucial role in NEPC development through complex interactions with multiple signaling pathways. The RE1 silencing transcription factor (REST), which normally suppresses neuronal gene expression in prostate epithelial cells, becomes inactivated under hypoxic conditions. This inactivation occurs through multiple mechanisms, including destabilization of the REST protein following AR inhibition and conversion to its inactive isoform REST4 by the splicing factor serine/arginine repetitive matrix 4 (SRRM4) [71]. The loss of REST function leads to increased expression of neuroendocrine markers such as chromogranin A, synaptophysin, and AURKA [72]. Furthermore, REST suppression releases ONECUT2, a transcription factor that promotes neuroendocrine differentiation by activating PEG10 expression while inhibiting androgen receptor and FOXA1 signaling [73]. ONECUT2 also enhances hypoxic signaling through HIF-1 α activation via mothers against decapentaplegic homolog 3 (SMAD3), creating a feed-forward loop that further promotes neuroendocrine transformation [74, 75].

7. Novel therapeutic targets for NEPC

The prognosis for patients with NEPC is generally poor, with a reported median survival of 16.8 months for de novo NEPC, while t-NEPC demonstrates longer survival at 53.5 months from initial diagnosis [27]. Despite some advancements in the treatment landscape, there is still a significant need for new therapeutic strategies. First-line management still relies on combination chemotherapy with cisplatin and etoposide, a regimen also used to treat SCLC, given the morphological similarities between the two tumor identities [76].

Recent developments in NEPC treatment have explored immunotherapy approaches, particularly checkpoint inhibitors, which have shown efficacy against SCLC and other extrapulmonary neuroendocrine tumors [77, 78]. Prostate cancer remains an immune-cold tumor with sparse leukocyte infiltration. Therefore, the trials with immune checkpoint inhibitors have shown limited success, primarily benefiting rare cases with mismatch repair deficiency and microsatellite instability (MSI) [79, 80]. These patients were able to achieve notable responses to checkpoint blockade with pembrolizumab, while combining atezolizumab with standard chemotherapy does not appear to improve outcomes [81, 82].

Molecular similarities between aggressive prostate cancer variants and NEPC, such as typical alterations in RB1, TP53, and PTEN, suggest overlapping biology that may inform targeted treatment approaches [83]. PTEN loss leads to activation of the PI3K-Akt-mTOR pathway, making it a potential therapeutic target for agents such as everolimus [84]. Additionally, BRCA1 and BRCA2 mutations have been associated with aggressive prostate cancer forms and NEPC, with BRCA2 alterations found in approximately 29% of CRPC cases exhibiting the NEPC phenotype [85, 86]. In response, poly(ADP-ribose) polymerase (PARP) inhibitors have emerged as a promising treatment for tumors with BRCA mutations, showing efficacy in mCRPC [87]. However, the use of PARP inhibitors like olaparib in NEPC patients with BRCA1/2

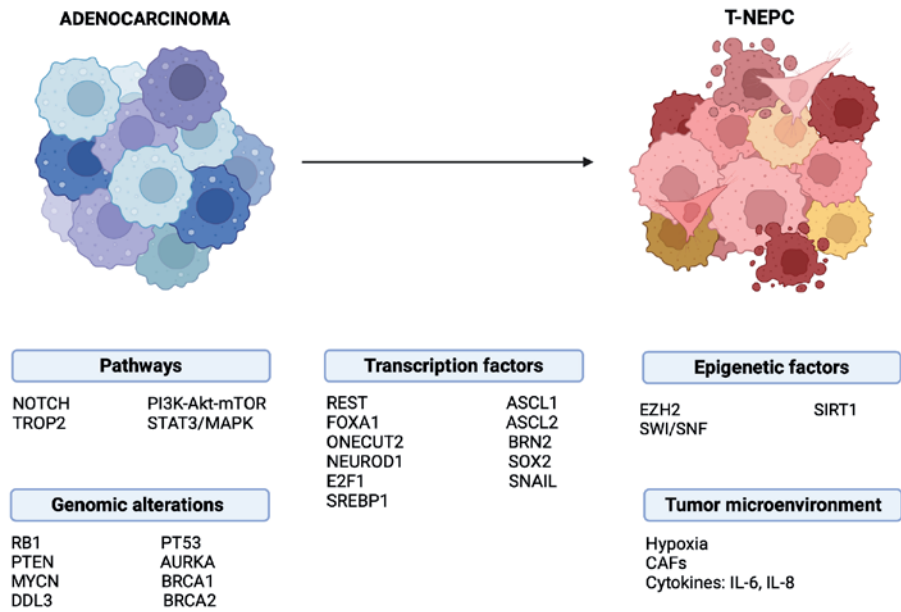


Figure 1.
Key factors contributing to NEPC transdifferentiation from prostate adenocarcinoma. Created with BioRender.

mutations has yielded mixed results. While some cases showed favorable responses, others reported adverse events or limited efficacy, which may be attributed to various mechanisms, including BRCA2 reverse mutations [88, 89]. Moreover, a small study comparing de novo and t-NEPC revealed a higher prevalence of germline DNA repair alterations in de novo NEPC compared to t-NEPC (about 11% vs. 2.5%) [27]. Recent studies have also explored combining PARP inhibitors with cyclin-dependent kinase inhibitors, showing potential in reducing neuroendocrine gene expression and tumor growth [90, 91].

Recent targeted therapies for NEPC focus on pathways involved in neuroendocrine differentiation. While a phase 2 trial of alisertib, an AURKA inhibitor, did not meet its primary endpoint, it identified four exceptional responders, suggesting potential for targeted approaches in select patients [92]. Another emerging therapeutic target is delta-like ligand 3 (DLL3), shown to be upregulated in NEPC [93]. Tarlatamab, a bispecific T-cell engager that binds DLL3 and CD4, is currently being evaluated in a phase 1b trial enrolling patients with de novo or treatment-emergent NEPC. Early data have indicated an overall response rate of 10.5%, with higher efficacy in DLL3-positive cases (Figure 1) [94].

8. Current challenges and emerging solutions

The research of NEPC faces significant challenges due to limited in vivo data that is attributed to the lack of standardized methods for developing NEPC models as well as the complexity of the disease. Patient-derived xenograft (PDX) models, while preserving tumor heterogeneity, often fail to replicate the dynamic progression from hormone-naïve prostate cancer (HNPC) to CRPC and NEPC. For

example, models like LTL331 transition to an NEPC phenotype (LTL331R) after castration but fail to fully capture the clinical transformation process [95]. The low success rate of generating NEPC-specific PDX models highlights the need for improved methodologies, such as simulating clinical conditions like androgen deprivation or using AR-targeted drugs to induce neuroendocrine transdifferentiation or employing clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) gene editing to introduce specific genetic alterations associated with NEPC development, such as RB1, TP53, and PTEN loss [96, 97].

The absence of reliable non-invasive biomarkers further complicates the identification of tumors transitioning from adenocarcinoma to neuroendocrine phenotypes. Recent research highlights a promising solution through liquid biopsy approaches, such as a circulating tumor cell (CTC)-based RNA assay. This method analyzed 116 longitudinal samples from 17 patients, including seven of them with confirmed NEPC. While the assay demonstrated moderate sensitivity (53%) and high specificity (91%) in single-sample testing, its accuracy improved to 100% when evaluating sequential samples, identifying all NEPC cases in patients over time. Elevated neuroendocrine CTC levels at baseline correlated with poorer clinical outcomes, underscoring their prognostic value [98]. Another innovative method uses cell-free DNA (cfDNA) methylation analysis and offers a non-invasive way to assess both tumor burden and neuroendocrine features through a targeted sequencing panel. Unlike traditional biopsies, this technique can capture the full spectrum of tumor heterogeneity, providing a more comprehensive view of the disease, as well as having the potential for early detection of neuroendocrine features. A key feature of this approach is the Phenotype Enrichment score, which successfully differentiates between adenocarcinoma and neuroendocrine variants of CRPC [99]. Recent studies have also revealed the significant presence of microRNA (miRNA) isoforms (isomiRs) in extracellular vesicles (EVs) from NEPC. These isomiRs may influence messenger RNA (mRNA) targeting and stability, potentially driving tumor progression. Notably, miR-92b isoforms, previously linked to brain tumors, were detected, suggesting unique roles in cancer biology. The study also distinguishes between t-NEPC and de novo NEPC subtypes, revealing divergent miRNA profiles that underscore their molecular differences [100]. These advancements represent a significant step toward more accurate, less invasive monitoring of advanced prostate cancer, potentially enabling earlier intervention and more personalized treatment strategies.

Other challenges for NEPC concern the limited treatment options as well as the lack of a standard second-line treatment option. Recent research has identified carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) as a promising therapeutic target for NEPC. This cell surface antigen is expressed in over 60% of NEPC cases across multiple patient cohorts while showing limited expression in normal tissues [101]. Preclinical studies using the CEACAM5-targeted antibody-drug conjugate labetuzumab govitecan have demonstrated complete responses in patient-derived CEACAM5-expressing tumors [102, 103]. Labetuzumab govitecan's design may provide both targeted and systemic anti-tumor effects, potentially addressing the challenge of tumor heterogeneity in NEPC [101]. These findings have led to plans for a phase I/II clinical trial of labetuzumab govitecan in patients with CEACAM5-positive NEPC, with potential applications in other CEACAM5-expressing types of NEPCs (**Table 1**).

Category	Emerging solution	Key features
Diagnostic tools	CTC-based RNA assay	• 53% sensitivity, 91% specificity in single samples • 100% accuracy with sequential sampling
	cfDNA methylation analysis	• Captures tumor heterogeneity • Phenotype Enrichment score for NEPC detection
	Extracellular vesicle miRNAs	• miRNA profiles for t-NEPC vs. de novo NEPC • Potential biomarkers for disease progression
Therapeutic targets	CEACAM5-targeted therapy	• Expressed in 60% of NEPC cases • Labetuzumab govitecan shows complete responses in preclinical studies
	Immunotherapy	• Limited success with checkpoint inhibitors • Effective in MSI-deficient cases • Pembrolizumab shows responses in select patients
	PI3K Akt–mTOR pathway	• Targeted by everolimus • Relevant in PTEN loss cases
	PARP inhibition	• Effective in BRCA1/2 mutated cases • Olaparib shows mixed results • Promising combinations with CDK inhibitors
	AURKA inhibition	• Phase II trial of alisertib • Did not meet the primary endpoint but identified exceptional responders
	DLL3-targeted therapies	• Tarlatamab as a bispecific T-cell engager • 10.5% overall response rate • Higher efficacy in DLL3-positive cases
	PDX models	• Androgen deprivation simulation • AR-targeted drug incorporation • CRISPR/Cas9 gene editing for NEPC-associated mutations

Table 1.
Summary for the emerging solutions for NEPC.

9. Conclusion

Significant advances have been made in our understanding of NEPC, including deeper insights into the complex molecular landscape and better characterization of the tumor microenvironment's role in disease progression. However, emerging diagnostic tools, as well as potential therapeutic targets, remain largely restricted to research settings rather than clinical practice. Moreover, despite our enhanced biological understanding, effective treatments for NEPC patients remain limited, and overall survival rates continue to be discouragingly low. The disconnect between our growing biological understanding and clinical outcomes underscores the urgent need for ongoing research and collaborative efforts to bridge the gap between basic science discoveries and the development of novel and effective treatments for patients with NEPC.

Author details

Nastasiia Artamonova and Isabel Heidegger*

Department of Urology, Medical University Innsbruck, Innsbruck, Austria

*Address all correspondence to: isabel-maria.heidegger@i-med.ac.at

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] Liu S, Alabi BR, Yin Q, Stoyanova T. Molecular mechanisms underlying the development of neuroendocrine prostate cancer. *Seminars in Cancer Biology*. 2022;**86**:57-68. Available from: <https://www.sciencedirect.com/science/article/pii/S1044579X22001183>
- [2] Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nature Medicine*. 2016;**22**:298-305
- [3] Zou M, Toivanen R, Mitrofanova A, Floch N, Hayati S, Sun Y, et al. Transdifferentiation as a mechanism of treatment resistance in a mouse model of castration-resistant prostate cancer. *Cancer Discovery*. 2017;**7**:736-749
- [4] Le TK, Duong QH, Baylot V, Fargette C, Baboudjian M, Colleaux L, et al. Castration-resistant prostate cancer: From uncovered resistance mechanisms to current treatments. *Cancers*. 2023;**15**(20):5047
- [5] Aparicio AM, Harzstark AL, Corn PG, Wen S, Araujo JC, Tu SM, et al. Platinum-based chemotherapy for variant castrate-resistant prostate cancer. *Clinical Cancer Research*. 2013;**19**:3621-3630. DOI: 10.1158/1078-0432.CCR-12-3791
- [6] Huang J, Yao JL, Sant'Agnese PA D, Yang Q, Bourne PA, Na Y. Immunohistochemical characterization of neuroendocrine cells in prostate cancer. *Prostate*. 2006;**66**:1399-1406
- [7] Huang J, Wu C, Sant'Agnese PA D, Yao JL, Cheng L, Na Y. Function and molecular mechanisms of neuroendocrine cells in prostate cancer. *Analytical and Quantitative Cytology and Histology*. 2007;**29**:128-138
- [8] Huang YH, Zhang YQ, Huang JT. Neuroendocrine cells of prostate cancer: Biologic functions and molecular mechanisms. *Asian Journal of Andrology*. 2019;**21**:291-295
- [9] Park JW, Lee JK, Witte ON, Huang J. FOXA2 is a sensitive and specific marker for small cell neuroendocrine carcinoma of the prostate. *Modern Pathology*. 2017;**30**:1262-1272. DOI: 10.1038/modpathol.2017.44
- [10] Huang J, Yao JL, Zhang L, Bourne PA, Quinn AM, Sant'Agnese PA D, et al. Differential expression of interleukin-8 and its receptors in the neuroendocrine and non-neuroendocrine compartments of prostate cancer. *The American Journal of Pathology*. 2005;**166**:1807-1815
- [11] Netto GJ, Amin MB, Berney DM, Compérat EM, Gill AJ, Hartmann A, et al. The 2022 World Health Organization classification of tumors of the urinary system and male genital organs-part B: Prostate and urinary tract tumors. *European Urology*. 2022;**82**:469-482
- [12] Wang W, Epstein JI. Small cell carcinoma of the prostate. A morphologic and immunohistochemical study of 95 cases. *The American Journal of Surgical Pathology*. 2008;**32**:65-71
- [13] Yao JL, Madeb R, Bourne P, Lei J, Yang X, Tickoo S, et al. Small cell carcinoma of the prostate: An immunohistochemical study. *The American Journal of Surgical Pathology*. 2006;**30**:705-712
- [14] Epstein JI, Amin MB, Beltran H, Lotan TL, Mosquera JM, Reuter VE, et al. Proposed morphologic classification of prostate cancer with neuroendocrine

- differentiation. *The American Journal of Surgical Pathology*. 2014;**38**:756-767
- [15] Giordano S, Tolonen T, Tolonen T, Hirsimäki S, Kataja V. A pure primary low-grade neuroendocrine carcinoma (carcinoid tumor) of the prostate. *International Urology and Nephrology*. 2010;**42**:683-687
- [16] Freschi M, Colombo R, Naspro R, Rigatti P. Primary and pure neuroendocrine tumor of the prostate. *European Urology*. 2004;**45**:166-170
- [17] Sun Y, Niu J, Huang J. Neuroendocrine differentiation in prostate cancer. *American Journal of Translational Research*. 2009;**1**:148-162
- [18] Humphrey PA. Histological variants of prostatic carcinoma and their significance. *Histopathology*. 2012;**60**:59-74
- [19] Evans AJ, Humphrey PA, Belani J, van der Kwast TH, Srigley JR. Large cell neuroendocrine carcinoma of prostate: A clinicopathologic summary of 7 cases of a rare manifestation of advanced prostate cancer. *The American Journal of Surgical Pathology*. 2006;**30**:684-693
- [20] Tu X, Chang T, Nie L, Qiu S, Xu H, Huang Y, et al. Large cell neuroendocrine carcinoma of the prostate: A systematic review and pooled analysis. *Urologia Internationalis*. 2019;**103**:383-390
- [21] Nguyen N, Franz RD, Mohammed O, Huynh R, Son CK, Khan RN, et al. A systematic review of primary large cell neuroendocrine carcinoma of the prostate. *Frontiers in Oncology*. 2024;**14**:1-8
- [22] Tamas EF, Epstein JI. Prognostic significance of paneth cell-like neuroendocrine differentiation in adenocarcinoma of the prostate. *The American Journal of Surgical Pathology*. 2006;**30**:980-985
- [23] Bakht MK, Derecichei I, Li Y, Ferraiuolo RM, Dunning M, Oh SW, et al. Neuroendocrine differentiation of prostate cancer leads to PSMA suppression. *Endocrine-Related Cancer*. 2018;**26**:131-146
- [24] Hofman MS, Violet J, Hicks RJ, Ferdinandus J, Thang SP, Akhurst T, et al. [(177)Lu]-PSMA-617 radionuclide treatment in patients with metastatic castration-resistant prostate cancer (LuPSMA trial): A single-Centre, single-arm, phase 2 study. *The Lancet Oncology*. 2018;**19**:825-833
- [25] Zhang Q, Han Y, Zhang Y, Liu D, Ming J, Huang B, et al. Treatment-emergent neuroendocrine prostate cancer: A clinicopathological and immunohistochemical analysis of 94 cases. *Frontiers in Oncology*. 2021;**10**:1-9
- [26] Han W, Rexiati N, Yu F, Wang Y. A case of de novo neuroendocrine prostate cancer presented with elevated level of serum CEA carrying BRCA2 mutation: Case report and literature review. *Frontiers in Oncology*. 2025:1-6
- [27] Conteduca V, Oromendia C, Eng KW, Bareja R, Sigouros M, Molina A, et al. Clinical features of neuroendocrine prostate cancer. *European Journal of Cancer*. 2019;**121**:7-18. DOI: 10.1016/j.ejca.2019.08.011
- [28] Chen J, Shi M, Chuen Choi SY, Wang Y, Lin D, Zeng H, et al. Genomic alterations in neuroendocrine prostate cancer: A systematic review and meta-analysis. *BJUI compass*. 2023;**4**:256-265
- [29] Chedgy EC, Vandekerkhove G, Herberts C, Annala M, Donoghue AJ, Sigouros M, et al. Biallelic tumour suppressor loss and DNA repair

defects in de novo small-cell prostate carcinoma. *The Journal of Pathology*. 2018;**246**:244-253

[30] Ku SY, Rosario S, Wang Y, Mu P, Seshadri M, Goodrich ZW, et al. Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. *Science*. 2017;**355**:78-83

[31] Mu P, Zhang Z, Benelli M, Karthaus WR, Hoover E, Chen CC, et al. SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer. *Science*. 2017;**355**:84-88

[32] Ida A, Okubo Y, Kasajima R, Washimi K, Sato S, Yoshioka E, et al. Clinicopathological and genetic analyses of small cell neuroendocrine carcinoma of the prostate: Histological features for accurate diagnosis and toward future novel therapies. *Pathology - Research and Practice*. 2022;**229**:153731. Available from: <https://www.sciencedirect.com/science/article/pii/S0344033821003927>

[33] Pearson HB, Li J, Meniel VS, Fennell CM, Waring P, Montgomery KG, et al. Identification of Pik3ca mutation as a genetic driver of prostate cancer that cooperates with Pten loss to accelerate progression and castration-resistant growth. *Cancer Discovery*. 2018;**8**:764-779. DOI: 10.1158/2159-8290.CD-17-0867

[34] Lee DK, Liu Y, Liao L, Li W, Danielpour D, Xu J. Neuroendocrine prostate carcinoma cells originate from the p63-expressing basal cells but not the pre-existing adenocarcinoma cells in mice. *Cell Research*. 2019;**29**:420-422

[35] Chen H, Sun Y, Wu C, Magyar CE, Li X, Cheng L, et al. Pathogenesis of prostatic small cell carcinoma involves the inactivation of the P53

pathway. *Endocrine-Related Cancer*. 2012;**19**:321-331

[36] Lotan TL, Gupta NS, Wang W, Toubaji A, Haffner MC, Chaux A, et al. ERG gene rearrangements are common in prostatic small cell carcinomas. *Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology*. 2011;**24**:820-828

[37] Aggarwal R, Huang J, Alumkal JJ, Zhang L, Feng FY, Thomas GV, et al. Clinical and genomic characterization of treatment-emergent small-cell neuroendocrine prostate cancer: A multi-institutional prospective study. *Journal of Clinical Oncology Official Journal of the American Society of Clinical Oncology*. 2018;**36**:2492-2503

[38] Vellky JE, Ricke WA. Development and prevalence of castration-resistant prostate cancer subtypes. *Neoplasia*. 2020;**22**:566-575. Available from: <https://www.sciencedirect.com/science/article/pii/S1476558620301482>

[39] Fraser JA, Sutton JE, Tazayoni S, Bruce I, Poole AV. hASH1 nuclear localization persists in neuroendocrine transdifferentiated prostate cancer cells, even upon reintroduction of androgen. *Scientific Reports*. 2019;**9**:19076

[40] Beltran H, Rickman DS, Park K, Chae SS, Sboner A, MacDonald TY, et al. Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. *Cancer Discovery*. 2011;**1**:487-495. DOI: 10.1158/2159-8290.CD-11-0130

[41] Otto T, Horn S, Brockmann M, Eilers U, Schüttrumpf L, Popov N, et al. Stabilization of N-Myc is a critical function of Aurora a in human neuroblastoma. *Cancer Cell*. 2009;**15**: 67-78. DOI: 10.1016/j.ccr.2008.12.005

- [42] Dardenne E, Beltran H, Benelli M, Gayvert K, Berger A, Puca L, et al. N-Myc induces an EZH2-mediated transcriptional program driving neuroendocrine prostate cancer. *Cancer Cell*. 2016;**30**:563-577
- [43] Li Z, Sun Y, Chen X, Squires J, Nowroozizadeh B, Liang C, et al. p53 mutation directs AURKA overexpression via miR-25 and FBXW7 in prostatic small cell neuroendocrine carcinoma. *Molecular Cancer Research*. 2015;**13**:584-591. DOI: 10.1158/1541-7786.MCR-14-0277-T
- [44] Zhang XW, Li JY, Li L, Hu WQ, Tao Y, Gao WY, et al. Neurokinin-1 receptor drives PKC α -AURKA/N-Myc signaling to facilitate the neuroendocrine progression of prostate cancer. *Cell Death & Disease*. 2023;**14**:1-14
- [45] Hsu EC, Rice MA, Bermudez A, Marques FJG, Aslan M, Liu S, et al. Trop2 is a driver of metastatic prostate cancer with neuroendocrine phenotype via PARP1. *Proceedings of the National Academy of Sciences of the United States of America*. 2020;**117**:2032-2042
- [46] Han J, Li E, Chen L, Zhang Y, Wei F, Liu J, et al. The CREB coactivator CRTC2 controls hepatic lipid metabolism by regulating SREBP1. *Nature*. 2015;**524**:243-246
- [47] Gouw AM, Margulis K, Liu NS, Raman SJ, Mancuso A, Toal GG, et al. The MYC oncogene cooperates with sterol-regulated element-binding protein to regulate lipogenesis essential for neoplastic growth. *Cell Metabolism*. 2019;**30**:556-572.e5
- [48] Xiao L, Tien JC, Vo J, Tan M, Parolia A, Zhang Y, et al. Epigenetic reprogramming with antisense oligonucleotides enhances the effectiveness of androgen receptor inhibition in castration-resistant prostate cancer. *Cancer Research*. 2018;**78**:5731-5740. DOI: 10.1158/0008-5472.CAN-18-0941
- [49] Kufe DW. Emergence of MUC1 in mammals for adaptation of barrier epithelia. *Cancers*. 2022;**14**(1):1-12
- [50] Kufe DW. MUC1-C in chronic inflammation and carcinogenesis; emergence as a target for cancer treatment. *Carcinogenesis*. 2020;**41**:1173-1183. DOI: 10.1093/carcin/bgaa082
- [51] Kufe D. Dependence on MUC1-C in progression of neuroendocrine prostate cancer. *International Journal of Molecular Sciences*. 2023;**24**:1-13
- [52] Bhattacharya A, Fushimi A, Yamashita N, Hagiwara M, Morimoto Y, Rajabi H, et al. MUC1-C dictates JUN and BAF-mediated chromatin remodeling at enhancer signatures in cancer stem cells. *Molecular Cancer Research*. 2022;**20**:556-567
- [53] Yasumizu Y, Rajabi H, Jin C, Hata T, Pitroda S, Long MD, et al. MUC1-C regulates lineage plasticity driving progression to neuroendocrine prostate cancer. *Nature Communications*. 2020;**11**:338. DOI: 10.1038/s41467-019-14219-6
- [54] Rubin MA, Bristow RG, Thienger PD, Dive C, Imielinski M. Impact of lineage plasticity to and from a neuroendocrine phenotype on progression and response in prostate and lung cancers. *Molecular Cell*. 2020;**80**:562-577. DOI: 10.1016/j.molcel.2020.10.033
- [55] Balanis NG, Sheu KM, Esedebe FN, Patel SJ, Smith BA, Park JW, et al. Pan-cancer convergence to a small-cell neuroendocrine phenotype that shares susceptibilities with hematological malignancies. *Cancer Cell*. 2019;**36**:17-34.e7. DOI: 10.1016/j.ccell.2019.06.005

- [56] Cejas P, Xie Y, Font-Tello A, Lim K, Syamala S, Qiu X, et al. Subtype heterogeneity and epigenetic convergence in neuroendocrine prostate cancer. *Nature Communications*. 2021;**12**:5771. DOI: 10.1038/s41467-021-26042-z
- [57] Rodarte KE, Nir Heyman S, Guo L, Flores L, Savage TK, Villarreal J, et al. Neuroendocrine differentiation in prostate cancer requires ASCL1. *Cancer Research*. 2024;**84**:3522-3537
- [58] Chen CC, Tran W, Song K, Sugimoto T, Obusan MB, Wang L, et al. Temporal evolution reveals bifurcated lineages in aggressive neuroendocrine small cell prostate cancer trans-differentiation. *Cancer Cell*. 2023;**41**:2066-2082.e9. DOI: 10.1016/j.ccell.2023.10.009
- [59] Tao L, Huang G, Song H, Chen Y, Chen L. Cancer associated fibroblasts: An essential role in the tumor microenvironment. *Oncology Letters*. 2017;**14**:2611-2620
- [60] Mishra R, Halder S, Placencio V, Madhav A, Rohena-Rivera K, Agarwal P, et al. Stromal epigenetic alterations drive metabolic and neuroendocrine prostate cancer reprogramming. *The Journal of Clinical Investigation*. 2018;**128**:4472-4484
- [61] Bhowmick N, Posadas E, Ellis L, Freedland SJ, Di VD, Freeman MR, et al. Targeting glutamine metabolism in prostate cancer. *Frontiers in Bioscience-Elite*. 2023;**15**:1-15
- [62] Spiotto MT, Chung TD. STAT3 mediates IL-6-induced neuroendocrine differentiation in prostate cancer cells. *Prostate*. 2000;**42**:186-195
- [63] Ishii K, Sasaki T, Iguchi K, Kajiwarra S, Kato M, Kanda H, et al. Interleukin-6 induces VEGF secretion from prostate cancer cells in a manner independent of androgen receptor activation. *Prostate*. 2018;**78**:849-856
- [64] Kim J, Jin H, Zhao JC, Yang YA, Li Y, Yang X, et al. FOXA1 inhibits prostate cancer neuroendocrine differentiation. *Oncogene*. 2017;**36**:4072-4080
- [65] DaSilva JO, Amorino GP, Casarez EV, Pemberton B, Parsons SJ. Neuroendocrine-derived peptides promote prostate cancer cell survival through activation of IGF-1R signaling. *Prostate*. 2013;**73**:801-812
- [66] Amorino GP, Parsons SJ. Neuroendocrine cells in prostate cancer. *Critical Reviews in Eukaryotic Gene Expression*. 2004;**14**:287-300
- [67] Gackière F, Bidaux G, Delcourt P, Van Coppenolle F, Katsogiannou M, Dewailly E, et al. CaV3.2 T-type calcium channels are involved in calcium-dependent secretion of neuroendocrine prostate cancer cells. *The Journal of Biological Chemistry*. 2008;**283**:10162-10173
- [68] Zhang Y, Chen B, Xu N, Xu P, Lin W, Liu C, et al. Exosomes promote the transition of androgen-dependent prostate cancer cells into androgen-independent manner through up-regulating the Heme Oxygenase-1. *International Journal of Nanomedicine*. 2021;**16**:315-327
- [69] Bhagirath D, Yang TL, Tabatabai ZL, Majid S, Dahiya R, Tanaka Y, et al. BRN4 is a novel driver of neuroendocrine differentiation in castration-resistant prostate cancer and is selectively released in extracellular vesicles with BRN2. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2019;**25**:6532-6545
- [70] Yamada Y, Beltran H. Clinical and biological features of neuroendocrine

prostate cancer. *Current Oncology Reports*. 2021;**23**:15

[71] Zhang X, Coleman IM, Brown LG, True LD, Kollath L, Lucas JM, et al. SRRM4 expression and the loss of REST activity may promote the emergence of the neuroendocrine phenotype in castration-resistant prostate cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2015;**21**:4698-4708

[72] Svensson C, Ceder J, Iglesias-Gato D, Chuan YC, Pang ST, Bjartell A, et al. REST mediates androgen receptor actions on gene repression and predicts early recurrence of prostate cancer. *Nucleic Acids Research*. 2014;**42**:999-1015

[73] Bradley CA. ONECUT2 many towards AR-independence. *Nature Reviews. Urology*. 2019;**16**:65

[74] Guo H, Ci X, Ahmed M, Hua JT, Soares F, Lin D, et al. ONECUT2 is a driver of neuroendocrine prostate cancer. *Nature Communications*. 2019;**10**:278

[75] Rotinen M, You S, Yang J, Coetzee SG, Reis-Sobreiro M, Huang WC, et al. ONECUT2 is a targetable master regulator of lethal prostate cancer that suppresses the androgen axis. *Nature Medicine*. 2018;**24**:1887-1898

[76] Nadal R, Schweizer M, Kryvenko ON, Epstein JI, Eisenberger MA. Small cell carcinoma of the prostate. *Nature Reviews. Urology*. 2014;**11**:213-219

[77] Zhang S, Cheng Y. Immunotherapy for extensive-stage small-cell lung cancer: Current landscape and future perspectives. *Frontiers in Oncology*. 2023;**13**:1-9

[78] Pan WX, Zhang XM, Hao SL, Han W. Progress in immunotherapy for neuroendocrine neoplasm of the

digestive system. *World Journal of Gastroenterology*. 2023;**29**:4174-4185

[79] Vitkin N, Nersesian S, Siemens DR, Koti M. The tumor immune contexture of prostate cancer. *Frontiers in Immunology*. 2019;**10**:603

[80] Stultz J, Fong L. How to turn up the heat on the cold immune microenvironment of metastatic prostate cancer. *Prostate Cancer and Prostatic Diseases*. 2021;**24**:697-717

[81] Yoshida T, Yaegashi H, Toriumi R, Kadomoto S, Iwamoto H, Izumi K, et al. Long response duration to pembrolizumab in metastatic, castration-resistant prostate cancer with microsatellite instability-high and neuroendocrine differentiation: A case report. *Frontiers in Oncology*. 2022;**12**:912490

[82] Wee CE, Costello BA, Orme JJ, Quevedo JF, Pagliaro LC. Chemotherapy with atezolizumab for small cell or neuroendocrine carcinoma of the prostate: A single institution experience. *Prostate*. 2021;**81**:938-943

[83] Aparicio AM, Shen L, Tapia ELN, Lu JF, Chen HC, Zhang J, et al. Combined tumor suppressor defects characterize clinically defined aggressive variant prostate cancers. *Clinical Cancer Research*. 2016;**22**:1520-1530

[84] Kmak JA, Agarwal N, He Y, Heilmann AM, Miller VA, Ross JS, et al. Exceptional response to everolimus in a patient with metastatic castrate-resistant prostate cancer harboring a PTEN inactivating mutation. *Case Reports in Oncology*. 2020;**13**:456-461. DOI: 10.1159/000506625

[85] Castro E, Goh C, Olmos D, Saunders E, Leongamornlert D, Tymrakiewicz M, et al. Germline BRCA

- mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer. *Journal of Clinical Oncology*. 2013;**31**:1748-1757
- [86] Beltran H, Hruszkewycz A, Scher HI, Hildesheim J, Isaacs J, Yu EY, et al. The role of lineage plasticity in prostate cancer therapy resistance. *Clinical Cancer Research*. 2019;**25**:6916-6924
- [87] Teyssonneau D, Dariane C, Barret E, Beauval JB, Brureau L, Fiard G, et al. PARP inhibitors in prostate cancers, is it time for combinations? *Therapeutic Advances in Medical Oncology*. 2024;**16**:17588359241242960. DOI: 10.1177/17588359241242959
- [88] Turina CB, Coleman DJ, Thomas GV, Fung AW, Alumkal JJ. Molecular testing identifies determinants of exceptional response and guides precision therapy in a patient with lethal, treatment-neuroendocrine prostate cancer. *Cureus*. 2019;**11**:e5197
- [89] Pandya D, Shah M, Kaplan F, Martino C, Levy G, Kazanjian M, et al. Treatment-emergent neuroendocrine prostate cancer with a germline BRCA2 mutation: Identification of a candidate reversion mutation associated with platinum/PARP-inhibitor resistance. *Cold Spring Harbor Molecular Case Studies*. 2021;**7**(1):a006295
- [90] Liu B, Li L, Yang G, Geng C, Luo Y, Wu W, et al. PARP inhibition suppresses GR-MYCNDK5-RB1-E2F1 signaling and neuroendocrine differentiation in castration-resistant prostate cancer. *Clinical Cancer Research*. 2019;**25**:6839-6851
- [91] Wu C, Peng S, Pilie PG, Geng C, Park S, Manyam GC, et al. PARP and CDK4/6 inhibitor combination therapy induces apoptosis and suppresses neuroendocrine differentiation in prostate cancer. *Molecular Cancer Therapeutics*. 2021;**20**:1680-1691
- [92] Beltran H, Oromendia C, Danila DC, Montgomery B, Hoimes C, Szmulewitz RZ, et al. A phase II trial of the Aurora kinase a inhibitor alisertib for patients with castration-resistant and neuroendocrine prostate cancer: Efficacy and biomarkers. *Clinical Cancer Research*. 2019;**25**:43-51. DOI: 10.1158/1078-0432.CCR-18-1912
- [93] Thoma C. Targeting DLL3 in neuroendocrine prostate cancer. *Nature Reviews. Urology*. 2019;**16**:330
- [94] Aggarwal RR, Rottey S, Bernard-Tessier A, Mellado-Gonzalez B, Kosaka T, Stadler WM, et al. Phase 1b study of tarlatamab in de novo or treatment-emergent neuroendocrine prostate cancer (NEPC). *Journal of Clinical Oncology*. 2024;**42**:5012-5012
- [95] Berman-Booty LD, Knudsen KE. Models of neuroendocrine prostate cancer. *Endocrine-Related Cancer*. 2015;**22**:R33-R49
- [96] Okasho K, Ogawa O, Akamatsu S. Narrative review of challenges in the management of advanced neuroendocrine prostate cancer. *Translational Andrology and Urology*. 2021;**10**:3953-3962
- [97] Cai H, Zhang B, Ahrenfeldt J, Joseph JV, Riedel M, Gao Z, et al. CRISPR/Cas9 model of prostate cancer identifies Kmt2c deficiency as a metastatic driver by Odam/Cabs1 gene cluster expression. *Nature Communications*. 2024;**15**:1023
- [98] Berchuck JE, Baca SC, McClure HM, Korthauer K, Tsai HK, Nuzzo PV, et al. Detecting neuroendocrine prostate cancer through tissue-informed cell-free

DNA methylation analysis. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2022;**28**:928-938

[99] Franceschini GM, Quaini O, Mizuno K, Orlando F, Ciani Y, Ku SY, et al. Noninvasive detection of neuroendocrine prostate cancer through targeted cell-free DNA methylation. *Cancer Discovery*. 2024;**14**:424-445

[100] Bhagirath D, Liston M, Akoto T, Lui B, Bensing BA, Sharma A, et al. Novel, non-invasive markers for detecting therapy induced neuroendocrine differentiation in castration-resistant prostate cancer patients. *Scientific Reports*. 2021;**11**:1-16. DOI: 10.1038/s41598-021-87441-2

[101] DeLucia DC, Cardillo TM, Ang L, Labrecque MP, Zhang A, Hopkins JE, et al. Regulation of CEACAM5 and therapeutic efficacy of an anti-CEACAM5-SN38 antibody-drug conjugate in neuroendocrine prostate cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2021;**27**:759-774

[102] Govindan SV, Cardillo TM, Moon SJ, Hansen HJ, Goldenberg DM. CEACAM5-targeted therapy of human colonic and pancreatic cancer xenografts with potent labetuzumab-SN-38 immunoconjugates. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2009;**15**:6052-6061

[103] Govindan SV, Cardillo TM, Rossi EA, Trisal P, McBride WJ, Sharkey RM, et al. Improving the therapeutic index in cancer therapy by using antibody-drug conjugates designed with a moderately cytotoxic drug. *Molecular Pharmaceutics*. 2015;**12**:1836-1847

Chapter 4

Molecular Mechanisms of Castration Resistance and the Current Clinical Landscape of CRPC Treatment

Soumya Basu, Akshita Saravanan, Roshni Matekar, Samiksha Bujone, Saanvi Vishnoi, Vishal Kumar Sahu, Subhayan Sur and Amit Ranjan

Abstract

Castrate-resistant prostate cancer (CRPC) is characterized by the persistence and progression of the disease in spite of androgen deprivation treatment (ADT), the standard practice for initial treatment for advanced or metastatic prostate cancer. The advancement of preexisting illness, the emergence of new metastases, or a persistent increase in serum prostate-specific antigen (PSA) levels are all signs of castrate-resistant prostate cancer. The persistence of androgen receptor signaling is the most widely recognized leading cause of CRPC. Consequently, current treatment approaches focus on targeting the androgen-dependent pathway, specifically the CYP17 enzyme, the androgen receptor (AR), and AR mRNA. Additional strategies include targeting androgen-independent pathways such as Src kinase, mTOR, and IGF-IR; apoptotic pathways through BCL-2; epigenetic pathways *via* HDAC inhibitors; and cell cycle disruption using specific CDK inhibitors. As tumor cells endure, they may develop into metastatic CRPC (mCRPC), typically leading to osteoblastic bone metastases. Current theranostic approaches for mCRPC primarily target the prostate-specific membrane antigen (PSMA) with radioligand therapy (RLT). This chapter aims to investigate the various molecular pathways associated with the development of castration resistance. It will assess the current clinical landscape by positioning existing strategies within the context of other available treatments. Additionally, it will address the challenges faced by a range of clinical drugs that are under investigation or currently in use for the treatment of CRPC.

Keywords: castrate-resistant prostate cancer (CRPC), metastasis, molecular pathways of castration resistance, theranostics, personalized medicine, clinical landscape, current treatments

1. Introduction

Prostate cancer (PCa) is the most commonly diagnosed malignancy world-wide and the sixth leading cause of cancer-related death in men [1]. According to one study by the American Cancer Society, there were an estimated 313,780 new cases and 35,770 deaths in the United States in 2025, although the majority of men diagnosed with PCa do not die from the disease [2]. In the United Kingdom, it is the second most common cause of male cancer death after lung cancer, similar to the situation in the United States [3]. In India, in males over 65, PCa (12.3%) is the second most common site of development after lung cancer (13.1%) [4]. Globally, PCa is the most commonly diagnosed malignancy in more than 50% of countries (112 out of 185) [5].

Although it only makes up 0.5% of all newly diagnosed cases in males under the age of 45 years, PCa is becoming more widespread in most nations across the world. The rise in prostate-specific antigen (PSA) testing, overdiagnosis, and previous underdiagnosis are presumably the cause of this statistics. Recent increases in obesity, metabolic syndrome, physical inactivity, HPV infections, chemical exposures, environmental carcinogenic exposures, and shifting referral patterns have been additional risk factors that might be playing a significant role [6]. Prostate tumors that continue to grow even when the body's testosterone levels are drastically lowered are known as castrate-resistant prostate cancers. CRPCs do not require normal testosterone levels to thrive, in contrast to many early-stage prostate cancers, and hence are also referred to as hormone-refractory or hormone-resistant prostate cancer [7]. Multiple mechanisms that enable prostate cancer cells to survive and multiply in spite of low amounts of circulating testosterone are part of the complex pathophysiology of CRPC. The phenomenon, known as intracrine and paracrine androgen production, is one important cause of this resistance. Intracrine production occurs when PCa cells may continue to manufacture and utilize androgens within the same cell, without secreting them into their surroundings. On the other hand, paracrine production involves PCa cells secreting androgens into the tumor microenvironment, where it can be accessed and used by neighboring PCa cells. This local synthesis of androgens persists even after pharmacological or surgical castration has decreased systemic testosterone levels, which leads to sustained AR signaling, therefore becoming a major cause in the development of castration resistance [8]. CRPC is clinically significant since it is linked to the disease's progression in spite of androgen deprivation therapy (ADT). This is indicated by the development of new metastases, the progression of preexisting disease, or an increase in serum levels of the PSA [9]. Patients with CRPC have a catastrophic prognosis, especially if they have metastatic disease, as their median overall survival rate is less than 2 years. Additionally, 90% of CRPC instances result in bone metastases, which can cause serious side effects such as pain, fractures, and compression of the spinal cord [10]. Since CRPC is still difficult to manage, research into efficient treatment methods must continue.

This chapter will mainly focus on the mechanisms that propel the development of CRPC, and the current clinical landscape for its management, assessing their safety and effectiveness. We will also evaluate theranostic strategies, such as radioligand therapy targeting prostate-specific membrane antigen (PSMA), in treating metastatic CRPC and discuss challenges involved in its management.

2. Molecular mechanisms of castration resistance

A common treatment strategy used for prostate cancer includes ADT, the goal of which is to reduce serum testosterone to “castrate” levels. This strategy creates a state of androgen deprivation that induces apoptosis in androgen-dependent tumor cells. However, on average of a few months to 2–3 years of ADT [11], PCa progresses as the tumor cells acquire an androgen-liberated state, causing CRPC. The AR is a ligand-inducible transcription factor consisting of four distinguishable domains: a polymorphic N-terminal domain (NTD), a DNA-binding domain (DBD), a small hinge region, and a C-terminal ligand binding domain (LBD). Primary ligands for the AR include dihydrotestosterone (DHT) and Testosterone (depicted in **Figure 1**) [12]. There are various mechanisms at play, leading to castration resistance; these can broadly be categorized as being either androgen receptor (AR)-dependent or AR-independent mechanisms, that, if not treated promptly, could ultimately lead to the progression of CRPC (depicted in **Figure 2**). Therefore, understanding these underlying molecular mechanisms holds the key to finding better treatments for managing CRPC.

2.1 Androgen receptor-dependent mechanisms

In the absence of a ligand, the AR associates with a chaperone complex in the cytoplasm called heat shock protein 90 (HSP90) and undergoes proteasome-mediated degradation. The binding of the androgens testosterone or DHT to the AR results in the dissociation of the AR-HSP complex, leading to its translocation to the

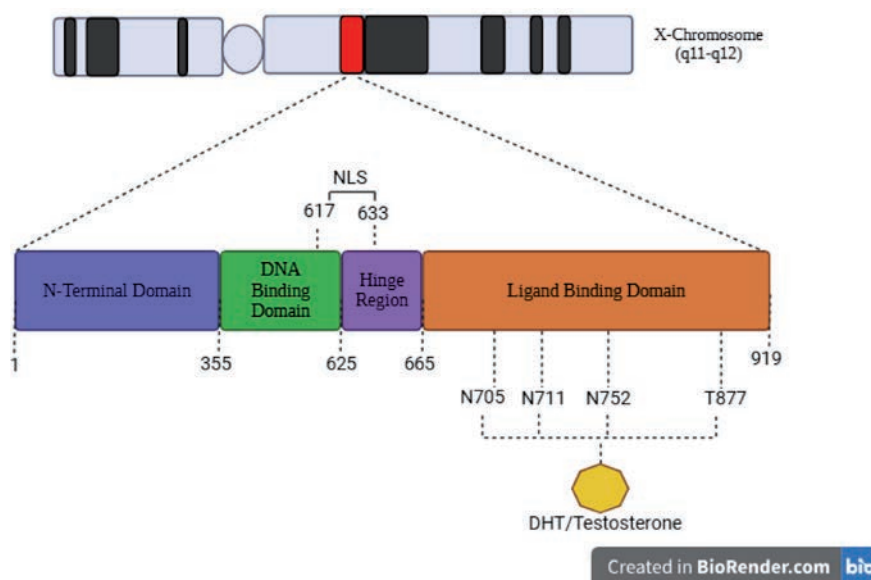


Figure 1. Androgen receptor protein- A linear depiction of the domain architecture of the androgen receptor (AR) protein, which is encoded by the X chromosome (q11–q12). The N-terminal domain (NTD), hinge region, ligand-binding domain (LBD), and DNA-binding domain (DBD) are all shown in the diagram. The nuclear localization signal (NLS) and important amino acid residues (N705, N711, N752, and T877) that are involved in DHT/testosterone binding are located within the LBD.

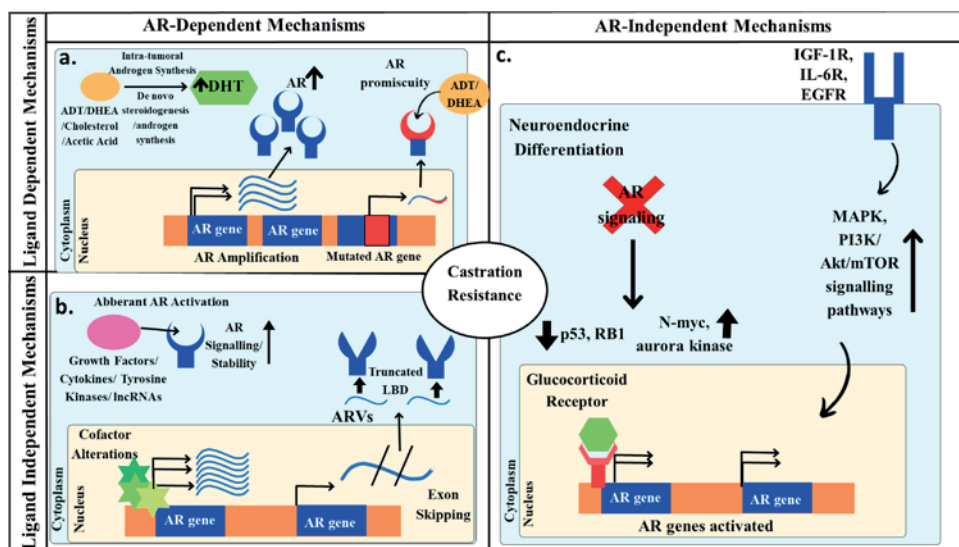


Figure 2.

Molecular mechanisms in the development of castration resistance in CRPC, depicting AR-dependent pathways involving (a) ligand-dependent mechanisms such as AR amplification, mutations in the AR gene, intra-tumoral androgen synthesis, and AR promiscuity, as well as (b) ligand-independent mechanisms such as AR splice variants, aberrant AR activation, and cofactor modifications, along with (c) AR-independent pathways including glucocorticoid receptor activation, neuroendocrine differentiation, and aberrant growth factor signaling.

nucleus and eventually homo-dimerization. In the nucleus, the AR dimer binds to androgen response elements (AREs) present in the promoter regions of target genes and recruits cofactors responsible for the regulation of androgen-regulated gene expression [13]. Several AR-dependent mechanisms have been identified that drive persistent androgen signaling in CRPC. These can be broadly classified into ligand-dependent mechanisms where AR signaling is induced due to the binding of a suitable ligand, such as Testosterone or DHT and ligand-independent mechanisms where the AR is activated through other signaling pathways.

2.1.1 Ligand-dependent mechanisms

2.1.1.1 AR amplification/overexpression

Overexpression of AR is often shown to occur from gene amplification, enhanced transcription-induced overexpression, and decreased degradation of AR mRNA. High levels of AR can result in hypersensitivity of the cell to the low residual amounts of ligand remaining after ADT (depicted in **Figure 2a**). Elevated AR protein levels and increased protein half-life and stability through E3 ligases such as MDM2 could also be contributors to the development of castration resistance [14, 15].

2.1.1.2 Mutation in the AR gene

The AR gene is located on the X chromosome at Xq11–12 and therefore is single-copy in males; thus, the phenotypic manifestation of mutations can occur without the influence of a wild-type codominant allele. Several point mutations have been shown to increase AR activity such as those occurring in the hinge regions have been shown to lead to an increase in AR transactivation activity (depicted in **Figure 2a**). The LBD

domain of the AR gene is a mutational hotspot; mutations in this region such as H8747, T877A and T877S, and T878A, relax ligand specificity allowing it to be activated by adrenal androgens and alternate steroidal hormones such as alpha-DHT and dehydroepiandrosterone (DHEA), estrogen, and progesterone [15]. The T878A mutation also results in antagonist-to-agonist switching. In the absence of this mutation, the drug behaves as intended, having antagonistic effects by blocking the activity of the receptor. However, the presence of this mutation results in an agonistic effect instead, where the binding of the drug causes receptor activation; therefore, treatment with AR antagonists flutamide and bicalutamide showed agonistic effects. Other such mutations that result in AR antagonist-to-agonist switching include L702H, W742C, A875U, W741C, and W741L mutations in the LBD encoding region [11].

2.1.1.3 Intra-tumoral androgen synthesis

Testosterone normally originates mainly from the testes or the adrenal glands. PCa cells may initiate the secretion of synthetic androgen in the prostate tissues. Possible mechanisms through which intra-tumoral androgen synthesis may occur could be through the conversion of adrenal androgens such as androstenedione and DHEA to testosterone, *de novo* androgen synthesis from acetic acid resulting in DHT or *de novo* steroidogenesis of androgen from cholesterol within these tumor tissues (depicted in **Figure 2a**) [14, 15].

2.1.2 Ligand-independent mechanisms

2.1.2.1 AR splice variants

AR splice variants (ARVs) arise due to alternate splicing of AR mRNA. These are characterized by truncation or exon skipping of the LBD region. In the presence of an intact LBD domain, the release of AR from HSP90 and subsequent nuclear translocation is induced *via* androgen-dependent regulation. ARVs lose this function due to the truncation of the LBD region of the protein (depicted in **Figure 2b**). However, despite the loss of functionality in binding, these truncated proteins remain active as transcription factors independently of androgen binding thus promoting the expression of target genes and activating downstream AR signaling pathways [16]. ARV1, ARV567, and ARV7 are predominantly expressed in CRPC with ARV7 being the most extensively studied variant. The ARV7 variant lacks the LBD, is located in the nucleus, and is constitutively active in regulating both AR-regulated genes and a certain subset of AR-independent genes. Some variants, such as the ARV8 variant lack the DBD, thus remaining constitutively active in the plasma membrane, activating cell signaling pathways such as AR phosphorylation via EGF-mediated SRC activation [17].

2.1.2.2 Aberrant AR activation

AR signaling and transcriptional activity are also increased by various growth factors, cytokines, and kinase pathways, without the need for binding of androgens, thus promoting progression to castration resistance in a ligand-independent manner (depicted in **Figure 2b**). Growth factors like IGF1, KGF, and EGF are also overexpressed in castrate-resistant tumors and can bind to and activate AR when serum androgen levels are at castrate states. Certain receptor tyrosine kinases such as HER-2/neu, EGFR, and IGF-1R too are overexpressed in this state, enhanced the stability and activity of AR. Cytokines such as IL-4 and IL-6 promote androgen independence. IL-6 activates

AR-mediated gene expression by activating AR through the Stat3 pathway [18]. NF- κ B signaling pathway also plays a role in the development of CRPC by promoting hyperplasia and inducing castration-resistant growth; this is achieved through limiting ADT-mediated apoptosis and cell cycle inhibition [17]. Two lncRNAs, PRNCR1 and PCGEM1, bind to the AR and enhance ligand-dependent and ligand-independent AR activation and consequent AR-mediated gene activation programs in prostate cancer cells [19].

2.1.2.3 Coactivator/corepressor modifications

AR, a transcription factor, recruits various coregulatory complexes that function as coactivators, enhancing, or corepressors, repressing its transcriptional activity. Many of these co-regulators are enzymes that modulate the activity of other proteins in that complex, acting as molecular chaperones, transcription machinery recruiters, or RNA splicing regulators. These co-regulators, however, facilitate the transcriptional activity of the AR even under androgen-depleted conditions, making these mechanisms a possible cause for the development of CRPC. CBP/P300, p160/SRC, FKBP51, and GATA2 are some coactivators of AR, which, when stimulated aberrantly, can increase PC growth through an increased transcriptional response (depicted in **Figure 2b**). Corepressors, on the other hand, are decreased in CRPC. The increase in the activity of coactivators and reduced control of corepressors causes an imbalance that results in persistent AR transcription activity in low levels of circulating androgen leading to an androgen-independent pro-growth state that facilitates CRPC [15, 17].

2.2 Androgen receptor independent mechanisms

2.2.1 Glucocorticoid receptor activation through bypass mechanism

Glucocorticoid receptors (GRs) and ARs have similar mechanisms of action, homologous DBDs, an overlapping transcriptome, and possess the same chromatin binding sites. Additionally, GRs also regulate the expression of a large number of AR-specific genes. Due to such high levels of homology, especially in the DBDs, activated GRs are able to mechanistically bind to nuclear APEs that regulate a subset of AR target genes that promote cell survival. Thus, an increase in expression and activity of GR may completely bypass the reliance on direct AR signaling (depicted in **Figure 2c**). An increase in the expression and activity of GR may therefore play a role in the progression of CRPC into metastatic CRPC [13, 20].

2.2.2 Neuroendocrine differentiation

Neuroendocrine cells residing within the normal prostate epithelium aid in its growth and differentiation, and lack AR expression. In prostate adenocarcinoma, the differentiation of AR-expressing cells to AR-negative neuroendocrine prostate cancer (NEPC) cells, termed lineage plasticity, is associated with castration resistance and the acquisition of a proliferative advantage under androgen-deprived conditions [21]. This phenotypic switch may be either *de novo* or treatment-related, such as due to prolonged hormonal manipulation or ADT. Important factors playing a role in NEPC differentiation include several mutations, such as loss of mutation in PTEN, p53 encoding the TP53 gene, and RB1. The amplification of the MYCN gene and the AURKA gene may also contribute to the gain of proliferative advantage in NEPC cells (depicted in **Figure 2c**) [22].

2.2.3 Growth factor and kinase pathways

Growth factor receptors such as IGF-1R, IL-6R, and EGFR can enhance the AR signaling pathway and mediate the activation of downstream pathways such as MAPK, promoting the growth of CRPC cells (depicted in **Figure 2c**). The activation of PI3K in the PI3K/Akt/mTOR signaling pathway leads to the phosphorylation of Akt, and subsequent activation of mTOR plays a significant role in the regulation of various cellular processes, such as cellular metabolism, differentiation, survival, migration, and angiogenesis. Loss of functionality of the PTEN gene or alterations in the PI3K and PTEN activities cause a loss of regulation of the Akt activity, thus leading to downstream upregulation of genes involved in cell survival and proliferation. It has also been shown to play a role in changes in AR expression and transcriptional activity [23]. Activation of Src kinases results in androgen-independent cell growth, migration, tumor invasion, adhesion, and inhibition of apoptotic signals, which may play a role in the progression into metastatic CRPC, especially in bone metastasis. Aberrant Src activation leads to the upregulation of the NF- κ B pathway of osteoclasts, due to which the cells may develop resistance to apoptosis signaling, and may result in enhanced cell proliferative properties thus leading to bone metastasis of prostate cancer [14].

3. Risk prediction

Numerous studies have examined the natural history of increased blood PSA in men with castration-resistant illness, emphasizing the importance of PSA dynamics in its prognosis [24]. In particular, PSA doubling time (PSA-DT), or the pace at which PSA increases, has become a crucial indicator of clinical results. Research has shown that in men with hormone-refractory prostate cancer, baseline PSA levels and PSA-DT are highly correlated with prostate cancer-specific mortality [25]. When CRPC is diagnosed, an elevated baseline PSA and a short PSA-DT are suggestive of aggressive disease and are linked to a markedly higher risk of death. Recent developments in computing, strategies, especially machine learning (ML), have improved the accuracy of CRPC risk prediction even more. ML models have outperformed conventional statistical techniques by utilizing sophisticated algorithms and big datasets [26]. To create individualized risk assessments, these models can incorporate a variety of clinical factors, such as PSA kinetics, Gleason score, and treatment history. Nevertheless, there are still issues with standardizing PSA-DT computations and confirming ML models across various demographics. To enhance CRPC risk prediction and customize treatment, future studies should combine advanced AI with genomic and imaging data. Emerging factors like PSMA and PSA long noncoding RNA are being actively explored for their potential to refine our understanding and prediction of CRPC.

4. Current treatment strategies for CRPC

Though ADT is the mainstay for PCa treatment, the development of castration resistance poses challenges in creating effective treatments. Current first-in-line treatment strategies target both AR-dependent and AR-independent mechanisms playing a role in castration resistance. Current treatment approaches for patients with nonmetastatic CRPC include supplementing ADT with AR antagonists such as enzalutamide, apalutamide, and darolutamide [27]. Guidelines set by the European

Association of Urology (EAU) for the treatment of mCRPC recommend the use of drugs such as docetaxel, abiraterone, enzalutamide, and sipuleucel-T, as first-line and cabazitaxel as second-line treatments [28].

4.1 Androgen receptor signaling inhibitors (ARSI)

AR signaling inhibitors (ARSI) are a class of drugs that interfere with AR signaling by either inhibiting the production of androgens through androgen synthesis inhibitors or blocking the activation of the AR through AR antagonists. Androgen synthesis inhibitors such as Ketoconazole, Orteronel, Galetrone, and Abiraterone acetate target androgen biosynthesis in testes, adrenal glands, and intratumoral regions, through inhibition of CYP17, an enzyme playing an essential role in the synthesis of adrenal and gonadal steroids [29]. Ketoconazole is a nonspecific cytochrome p450 inhibitor that shows androgen biosynthesis inhibition but has low potency and short half-life in the body, hence is obsolete for PC treatment [30, 31]. Orteronel (TAK-700) is a non-steroidal, selective, and reversible inhibitor of the 17,20-lyase activity of the CYP17A1 enzyme; reduces PSA levels; and suppresses androgen levels, causing shrinkage of androgen-dependent organs [32]. However, unsuccessful phase III clinical trials led to it being discontinued in 2014 [33]. Galeterone showed dual activity as an androgen biosynthesis inhibitor and an androgen antagonist, disrupting AR signaling through a proteasomal-dependent pathway, leading to AR degradation of both full-length AR and truncated ARVs [34]. However, ARMOR3-SV, a phase III trial, was unsuccessful in meeting its primary endpoint; thus was discontinued by Tokai Pharmaceuticals Inc. in 2016 [35]. Abiraterone acetate is the first FDA-approved second-generation antiandrogen widely used for treatment in PC. It was approved by the FDA in 2012 for treating patients with mCRPC [36]. It is usually given in combination with glucocorticoids such as prednisone to reduce side effects such as hypokalemia, edema, hepatotoxicity, and adrenocortical insufficiency [37]. In a study performed with abiraterone acetate, after a median follow-up of 30.4 months at a planned interim analysis (after 406 patients had died), the median overall survival was significantly longer in the abiraterone group than in the placebo group (not reached vs. 34.7 months) (hazard ratio for death, 0.62; 95% confidence interval [CI], 0.51 to 0.76; $P < 0.001$). The median length of radiographic progression-free survival was 33.0 months in the abiraterone group and 14.8 months in the placebo group (hazard ratio for disease progression or death, 0.47; 95% CI, 0.39 to 0.55; $P < 0.001$). Significantly better outcomes in all secondary end points were observed in the abiraterone group, including the time until pain progression, next subsequent therapy for prostate cancer, initiation of chemotherapy, and prostate-specific antigen progression ($P < 0.001$ for all comparisons), along with next symptomatic skeletal events ($P = 0.009$). The addition of abiraterone acetate and prednisone to androgen-deprivation therapy significantly increased overall survival and radiographic progression-free survival in men with newly diagnosed, metastatic, castration-sensitive prostate cancer [38].

First-generation nonsteroidal antiandrogens (flutamide, bicalutamide, and nilutamide) have limitations such as antagonist-to-agonist switching and low AR affinity resulting in incomplete and reversible transcriptional inhibition, highlighting the need for the development of potent second-generation antiandrogens. These potent AR antagonists include Enzalutamide, Apalutamide, and Darolutamide. Enzalutamide was a novel FDA-approved second-generation antiandrogen that did not exhibit agonistic behavior when used in an environment with AR overexpression [39].

It received FDA approval for the treatment of nmCRPC and mCRPC in 2018, based on the success of key trials PROSPER and AFFIRM [40]. Though clinically well-tolerated, high dosages of enzalutamide showed an increased risk of seizures [30]. In a study performed with enzalutamide and leuprolide, a total of 1068 patients underwent randomization: 355 were assigned to the combination group, 358 to the leuprolide-alone group, and 355 to the monotherapy group. The patients were followed for a median of 60.7 months. At 5 years, metastasis-free survival was 87.3% (95% confidence interval [CI], 83.0 to 90.6) in the combination group, 71.4% (95% CI, 65.7 to 76.3) in the leuprolide-alone group, and 80.0% (95% CI, 75.0 to 84.1) in the monotherapy group. With respect to metastasis-free survival, enzalutamide plus leuprolide was superior to leuprolide alone (hazard ratio for metastasis or death, 0.42; 95% CI, 0.30 to 0.61; $P < 0.001$); enzalutamide monotherapy was also superior to leuprolide alone (hazard ratio for metastasis or death, 0.63; 95% CI, 0.46 to 0.87; $P = 0.005$). In patients with prostate cancer with high-risk biochemical recurrence, enzalutamide plus leuprolide was superior to leuprolide alone with respect to metastasis-free survival; enzalutamide monotherapy was also superior to leuprolide alone [41].

Apalutamide, approved by the FDA in 2018 for nmCRPC following the key clinical trial SPARTAN, which showed significant improvement in metastasis-free survival [42], was found to be selective for AR over other nuclear hormone receptors and showed several advantages over Enzalutamide, namely, greater antitumor activity at lower doses, higher tumor-to-plasma ratio, and a decreased risk of seizure [39]. Darolutamide was approved by the FDA as a treatment option for nmCRPC in 2019, following the key clinical trial ARAMIS [43]. In competitive binding assays, it showed increased potency over enzalutamide and apalutamide [30]. EPI-7386 is a next-generation AR inhibitor that binds to the NTD of the AR and effectively blocks transcription despite AR resistance mechanisms driven by the LBD, such as point mutations and splice variants. However, this AR inhibitor is currently under clinical investigation and is yet to be approved by the FDA [44].

4.2 Cytotoxic chemotherapy

Cytotoxic chemotherapy remains a cornerstone in the management of castration resistance in PCa. Taxane-based chemotherapeutics, namely, docetaxel and cabazitaxel, have shown significant clinical benefits. Docetaxel is a semisynthetic taxane approved by the FDA in 2004 as a first-line treatment for mCRPC based on the results of a key phase III trial TAX327 [45]. Cabazitaxel is a novel second-generation semisynthetic taxane, showing improved survival in patients with docetaxel-refractory mCRPC [46, 47]. It was approved by the FDA in 2010 for treating mCRPC following the success of the key trial TROPIC [48].

4.3 Radiation-based therapies

Bone metastasis is a common complication in CRPC progression, affecting about 30% of patients within the first 2 years of the disease [49], leading to skeletal-related events (SREs) such as pathological bone fractures, spinal cord compression, or nerve root compression due to vertebral collapse, impairing patient mobility, quality of life, and survival [50].

Radiotherapy, particularly external beam radiation therapy (EBRT), is often recommended for the palliation of bone metastases [48] using various doses and fractionation schedules. Common schedules employed for pain relief include a single

fraction of 8 Gy and multiple fractions schedules including 30 Gy/10, 24 Gy/6 and 20 Gy/5. For those at risk of developing skeletal-related events, the 20 Gy/5 and 30 Gy/10 fractions can help downstage the tumor before surgery [51, 52].

Radiopharmaceuticals targeting bone metastasis can be used with treatment methods such as chemotherapy and EBRT or combined with bisphosphonates such as Zoledronic acid to enhance bone mineral density and reduce the incidence of total SREs [53]. Common bone metastasis targeting radiopharmaceuticals include [¹⁵³Sm]Sm-ethylene diamine tetramethylene phosphonate ([¹⁵³Sm]Sm-EDTMP), a β -emitter targeting osteoblastic bone metastases, approved by the FDA in 1993 [54]. Strontium-89 (Sr-89) is another β -emitter effective in reducing pain in patients with multiple bone metastases from CRPC. To increase the durability of response, these can be used in combination with EBRT or chemotherapy [51]. [²²³Ra] Radiumdichloride is the first FDA-approved α -particle-emitting agent used for the treatment of mCRPC patients exhibiting bone metastases with an absence of visceral metastases. Due to its calcium-mimetic properties, it is preferentially incorporated into areas with high bone turnover and induces local apoptosis of tumor cells [49]. Its short penetration range (<100 μ m) and high linear energy transfer (80 keV/ μ m) offer high efficacy with low myelosuppression [54]. The key clinical trial ALSYMPCA led to the FDA-approval of Ra-223 Dichloride (Xofigo) in 2013, for patients with CRPC showing symptomatic bone metastases with no known visceral metastasis [55].

4.4 Immunotherapy

Immunotherapy is an effective treatment approach for various malignancies, increasing survival rates and reducing tumor burden. However, the benefits from immunotherapies for PCa patients are limited as they exhibit low immunogenicity due to the presence of immune-suppressive cell populations in the prostate tumor microenvironment, impaired T-cell functionality, and MHC-I downregulation [55]. Some cancer therapeutic vaccines, such as Immune checkpoint inhibitors (ICIs) and active cellular immunotherapeutic vaccines have gained some success in the alleviation of CRPC.

ICIs include Ipilimumab, an anti-CTLA4 monoclonal antibody, Atezolizumab, a humanized antibody targeting programmed death protein ligand-1 (PD-L1) and Pembrolizumab, an anti-programmed death protein-1 (PD1) checkpoint inhibitor [54]. Currently, Pembrolizumab is the only FDA-approved ICI for prostate cancer. It was approved in 2017 for patients with high microsatellite instability, tumor mutational burden (TMB) ≥ 10 mut/Mb, and subsequent therapy for patients with resistance developed toward docetaxel and novel hormonal therapeutics [56]. Sipuleucel-T is the first FDA-approved active cellular immunotherapeutic cancer vaccine [9]. It gained FDA approval for treating asymptomatic mCRPC in 2010, following the success of the key phase III trial IMPACT [57]. A novel immunotherapeutic approach for CRPC treatment includes CAR-T cell therapy targeting a glycoprotein often expressed on the surface of PCa cells called Muc1 [58]. Denosumab (Xgeva) is another immunotherapeutic drug that was approved by the FDA in 2010 for treating mCRPC exhibiting bone metastasis [59].

5. Theranostics for metastatic CRPC

Theranostic drugs target disease-specific structures (such as proteins or antigens) that are expressed in the cancer cells of a patient or in the organ where

the disease has spread. For imaging and later treatment, these structures can be targets for ligands containing certain radioisotopes [60]. Typical first-line therapy for metastatic disease is a testosterone-reducing agent with androgen deprivation therapy (ADT), frequently combined with a novel androgen receptor (AR) axis inhibitor such as abiraterone or chemotherapy with docetaxel [61], to which the majority of patients have an initial response; however, with time, the disease progresses to mCRPC but interestingly remains dependent on the AR-signaling pathway [62]. Treatment approaches beyond traditional androgen deprivation therapy (ADT) and AR-targeted medications are required due to the rise of castrate-resistant prostate cancer (CRPC). Prostate-specific membrane antigen (PSMA)-based theranostic strategies present a promising treatment option for mCRPC. Theranostic medications can accomplish tailored radiation administration and accurate tumor imaging by taking advantage of PSMA's overexpression in prostate cancer cells.

5.1 Prostate-specific membrane antigen (PSMA) targeting

Prostate-specific membrane antigen (PSMA) is a type II transmembrane glycoprotein with 750 amino acids and folate hydrolase activity that was initially identified in 1993 [63]. Despite being expressed in healthy prostate tissue, its overexpression on the cell membrane of prostate cancer cells makes it a promising target for functional imaging [64]. Since there is no known natural ligand for PSMA, it is still unknown why it is elevated in prostate cancer. Nevertheless, PSMA constitutively internalizes PSMA ligands, which makes PSMA a desirable molecular and imaging target for prostate cancer [65]. Given that PSMA contributes to the cellular uptake of folate, it acts as a growth factor for cancerous cells. As a result, metabolically active cells express PSMA in high quantities. It is therefore a sign of disease activity and rises with higher Gleason scores [66].

The use of PSMA in primary staging is becoming more and more supported by research. Sixteen publications with 1309 patients were included in the meta-analysis, which found that the sensitivity and specificity were 86% [67].

5.1.1 Radioligand therapy (RLT) with PSMA-targeted radiopharmaceuticals

An important development in the treatment of metastatic castrate-resistant prostate cancer (mCRPC) is radioligand therapy (RLT), a type of nuclear medicine therapy, which uses radiopharmaceuticals that target the prostate-specific membrane antigen (PSMA), allowing direct delivery of radiation to the target cancer cells. By taking advantage of the high PSMA expression on PCa cells, this treatment approach minimizes off-target radiation exposure while delivering radioisotopes to tumor locations with selectivity. Typically, PSMA-targeted RLT uses ligands tagged with alpha-emitting radionuclides like actinium-225 (^{225}Ac) or beta-emitting radionuclides like lutetium-177 (^{177}Lu) [68]. These radionuclides cause double-strand breaks in DNA, which kill tumor cells. Clinical studies have shown that in individuals with mCRPC who have advanced on conventional treatments, ^{177}Lu -PSMA RLT can significantly increase PSA responses, enhance quality of life, and increase overall survival. Additionally, ^{225}Ac -PSMA RLT has the potential to target micrometastatic illness and overcome resistance to beta-emitting radionuclides due to its better linear energy transfer.

6. Combination therapy

Alternative therapeutic approaches are required due to the advent of castration resistance, even if ADT was initially effective in treating metastatic prostate cancer. Combination treatments that block both AR-dependent and AR-independent pathways are being researched in an effort to reduce tumor development and spread and increase patient survival. ADT and radiation therapy are being used to treat a growing number of high-risk patients in an effort to prevent or delay the onset of CR prostate cancer. Patients with intermediate- or high-risk illness are now strongly recommended to utilize ADT prior to, during, and following radiation therapy. When ADT and radiotherapy are combined, the rates of overall survival, disease-free survival, distant metastasis-free survival, and biochemical-free survival all rise in comparison to radiotherapy alone. Studies have showed that Muc1 CAR-T cells in combination with the antiandrogen flutamide effectively reduce prostate cancer tumor growth. Docetaxel chemotherapy in conjunction with ADT has been shown to improve prostate cancer survival in the GETUG-AFU 15 study (5.7-month increase) and the CHAARTED trial (13.6-month increase, 8.5-month longer progression-free survival) [58]. Combining PSMA-targeted RLT with AR signaling inhibitors like enzalutamide or abiraterone is one potential strategy for mCRPC treatment [69]. Combining immunotherapy, such as immune checkpoint drugs, with PSMA-targeted RLT is another area of interest. The idea behind this strategy is to strengthen the immune response against tumors by taking use of radiation's immunomodulatory properties.

Clinical trials are also investigating the combination of PSMA-targeted RLT with DNA damage response inhibitors, such as PARP inhibitors, in patients with homologous recombination repair gene mutations [70]. Additionally, the synergistic potential of PSMA-targeted RLT with EBRT or stereotactic body radiotherapy is being explored to improve local control and eradicate oligometastatic disease. Future research should prioritize the identification of optimal combination strategies, the development of predictive biomarkers for patient selection, and the evaluation of long-term outcomes in well-designed clinical trials (**Table 1**).

7. Future directions

7.1 Precision medicine approaches

The genetic basis of PCa has been implicated in a large portion of the disorder's variability. Targetable mutations were found in 19.3% of patients in a study of men with mCRPC, and about half of these variants were inherited. Precision oncology relies on tumor genetic profiling to identify actionable mutations and pathways for targeted therapies. While tissue biopsies are standard, liquid biopsies using circulating tumor DNA (ctDNA) are gaining traction, with clinical trials exploring their utility [92]. Mismatch repair deficiency (MMRd), often resulting in microsatellite instability (MSI) and high tumor mutation burden, is associated with loss of function in MLH1, MSH2, MSH6, or PMS2 genes. Approximately 3–5% of prostate cancers globally exhibit high MSI, primarily due to somatic mutations, as germline mutations are rare (0.2–0.7% in metastatic prostate cancer). Despite similar responses to standard treatments, MMRd, like homologous recombination repair (HRR) mutations, is linked to more aggressive disease [93]. The FDA's approval of PARP inhibitors for mCRPC patients with HRR-related gene mutations marked the

Sr. no.	Drug name	Mechanism of action	Key trials (phase III)	Patient population	Trial characteristics	Outcomes with respect to primary end points	References
ARSIIs							
1	Abiraterone acetate	Second-generation antiandrogen selectively and irreversibly inhibits CYP17 resulting in decreased androgen production downstream of CYP17 [71].	COUAA-301	1195 docetaxel-refractive mCRPC patients	Abiraterone acetate + prednisone vs. placebo + prednisone	Overall survival (OS) was longer in the abiraterone acetate + prednisone group than in the placebo + prednisone group (median OS = 14.8 vs. 10.9 months; HR = 0.65; 95% confidence interval (CI), 0.54 to 0.77; P < 0.001)	[72]
2			COUAA-302	1088 patients with chemotherapy-naïve mCRPC	Abiraterone acetate + prednisone vs. placebo + prednisone	OS improved in abiraterone acetate + prednisone group (median not reached vs. 27.2 months for prednisone alone; HR = 0.75; 95% CI, 0.61 to 0.93; P = 0.01)	[73]
Side effects:		Side effects include hypokalemia, edema, hepatotoxicity, and adrenocortical insufficiency.					[37]
3	Enzalutamide	Second-generation antiandrogen with a selectivity for AR over other nuclear receptors, inhibits nuclear translocation, coactivator peptide recruitment and alters the DBD of AR by inducing a conformational change in the AR [30].	AFFIRM	1199 patients with CRPC pre-treated with docetaxel-based chemotherapy	Enzalutamide vs. placebo	The median OS was 18.4 months in enzalutamide group (95% CI, 17.3 to not yet reached) vs. 13.6 months in the placebo group (95% CI, 11.3 to 15.8) (HR for death in the enzalutamide group, 0.63; 95% CI, 0.53 to 0.75; P < 0.001)	[74]

Sr. no.	Drug name	Mechanism of action	Key trials (phase III)	Patient population	Trial characteristics	Outcomes with respect to primary end points	References
4			PREVAIL	1717 patients with chemotherapy-naïve metastatic PCa	Enzalutamide vs. placebo	The rate of radiographic progression-free survival at 12 months was 65% among patients treated with enzalutamide, as compared with 14% among patients receiving placebo (HR in the enzalutamide group = 0.19; 95% CI, 0.15 to 0.23; P < 0.001)	[75]
5			PROSPER	1401 patients with nmCRPC	Enzalutamide + ADT vs. placebo + ADT	Median OS was 67.0 months (95% CI, 64.0 to not reached) in the enzalutamide group and 56.3 months (95% CI, 54.4 to 63.0) in the placebo group HR for death (0.73; 95% CI, 0.61 to 0.89; P = 0.001).	[76]
Side effects:		A major challenge with enzalutamide treatment is the development of resistance, limiting its long-term effectiveness. Side effects include hypertension, fatigue, and cognitive issues, complicating its use in patients with comorbidities. The drug may have off-target effects impacting receptors apart from the prostate cancer ARs intended to target and responses can differ based on tumor molecular characteristics. Moreover, mutations in the AR such as the F876L mutation can further contribute to resistance, limiting its effectiveness in PCa treatment.					[77]
6	Apalutamide	Synthetic compound generated using structural activity relation (SAR) guided chemistry, showing higher affinity to the LBD of AR and inhibits its translocation to nucleus, and consequent binding to the DNA [30].	SPARTAN	1207 patients with nmCRPC	Apalutamide vs. placebo	Median metastasis-free survival was 40.5 months in apalutamide group as compared with 16.2 months in the placebo group (HR for metastasis or death, 0.28; 95% CI, 0.23 to 0.35; P < 0.001)	[78]

Sr. no.	Drug name	Mechanism of action	Key trials (phase III)	Patient population	Trial characteristics	Outcomes with respect to primary end points	References
Side effects:		Apart from off target effects, and the development of resistance against the drug, the most common side effects associated with the use of apalutamide were mild macular or maculopapular skin rash, fatigue, hypertension, hypothyroidism, arthralgia, and an increased risk of fractures.					[79]
7	Darolutamide	Synthetically generated, specifically inhibits AR nuclear translocation. Especially inhibiting AR mutants such as ARF876L that trigger enzalutamide and apalutamide antagonist to agonist switch, leading to decreased chances of therapeutic resistance development [30].	ARAMIS	1509 patients with nmCRPC	Darolutamide vs. placebo	Median metastasis-free survival was 40.4 months with darolutamide, as compared with 18.4 months with placebo (HR for metastasis or death in the darolutamide group, 0.41; 95% confidence interval, 0.34 to 0.50; P < 0.001)	[80]
Side effects:		Side effects included fatigue, increased risk of fracture, rash, and hypertension.					[81]
Chemotherapeutics							
1	Docetaxel	Semisynthetic taxane that stabilizes microtubules by binding to free tubulin within cells and preventing depolymerization. This results in the inhibition of mitosis due to the accumulation of microtubules and apoptosis through the phosphorylation of BCL-2, may also interfere with AR trafficking [82]	TAX 327	1006 men with metastatic hormone-resistant prostate cancer (mHRPC)	Docetaxel + prednisone (administered weekly) vs. mitoxantrone + prednisone (administered once every 3 weeks) vs. docetaxel + prednisone (administered once every 3 weeks)	Median OS was 16.5 months in the mitoxantrone group, 18.9 months in the group given docetaxel every 3 weeks, and 17.4 months in the group given weekly docetaxel. As compared to the mitoxantrone group, docetaxel group (administration every 3 weeks) had an HR for death of 0.76 (95% CI, 0.62 to 0.94; P = 0.009) and those given docetaxel (weekly administration) had an HR for death of 0.91 (95% CI, 0.75 to 1.11; P = 0.36).	[83]

Sr. no.	Drug name	Mechanism of action	Key trials (phase III)	Patient population	Trial characteristics	Outcomes with respect to primary end points	References
Side effects:		Side effects included fatigue, musculoskeletal or nail changes, sensory neuropathy, neutropenia, and febrile neutropenia.					[83]
2	Cabazitaxel	Novel second-generation semisynthetic tubulin-binding taxan with a similar mechanism of action to docetaxel, however, it has less affinity for multidrug resistance proteins such as the P-glycoprotein efflux pump [46].	TROPIC	755 patients with docetaxel-resistant mCRPC	Mitoxantrone + prednisone vs. cabazitaxel + prednisone	Median OS was 15.1 months in the cabazitaxel + prednisone group (95% CI 14.1 to 16.3) and 12.7 months (11.6 to 13.7) in the mitoxantrone + prednisone group. HR for death was 0.70 (95% CI 0.59–0.83, $P < 0.0001$)	[84]
3			CARD	255 patients with mCRPC pretreated with docetaxel showing progression within 12 months after receiving ARSIs (enzalutamide/abiraterone acetate)	Cabazitaxel + prednisone vs. enzalutamide/abiraterone acetate + prednisone	Median imaging-based progression-free survival was 8.0 months in the cabazitaxel group and 3.7 months in the ARSI. Imaging-based progression or death was reported in 73.6% of the cabazitaxel group, as compared 80.2% in the ARSI group (HR, 0.54; 95% CI, 0.40 to 0.73; $P < 0.001$)	[85]

Sr. no.	Drug name	Mechanism of action	Key trials (phase III)	Patient population	Trial characteristics	Outcomes with respect to primary end points	References
Side effects:		Side effects include febrile neutropenia, rash, fatigue, diarrhea, and alopecia.					[86]
Immunotherapeutics							
1	Pembrolizumab	Anti-programmed death protein-1 (PD1) checkpoint inhibitor that amplifies the T-cell mediated response by blocking PD1 signaling therefore prolonging T-cell activation and restoring T-cell proliferation [87]	KEYNOTE-199 (phase II trial)	A total of 388 patients with mCRPC, pretreated with docetaxel-based chemotherapy belonging to three cohorts: Cohort 1 with RECIST-measurable PD-L1-positive, Cohort 2 with RECIST-measurable PD-L1-negative disease and Cohort 3 with bone-predominant disease	Pembrolizumab is administered every 3 weeks to patients belonging to all three cohorts	Median OS was 9.5 months in cohort 1, 7.9 months in cohort 2, and 14.1 months in cohort 3. Objective response rate was 5% (95% CI, 2–11% of patients) in cohort 1 and 3% (95% CI, <1–11% of patients) in cohort 2. Disease control rate was 10% in cohort 1, 9% in cohort 2, and 22% in cohort 3.	[88]
Side effects:		Side effects included fatigue, diarrhea, decreased appetite, and colitis. Other immune-related side effects included hypothyroidism, hyperthyroidism, pneumonitis, and severe skin reactions.					[88]
2	Sipuleucel-T	Active cellular immunotherapeutic cancer vaccine: autologous PBMCs, (APCs) primed ex-vivo with PA2024, a recombinant fusion protein consisting of prostate antigen, prostatic acid phosphatase, fused to granulocyte-macrophage colony-stimulating factor.	IMPACT (Phase III trial)	512 patients with mCRPC	Sipuleucel-T vs. APC-Placebo	Relative reduction of 22% in the risk of death in the sipuleucel-T group as compared with the placebo group (HR, 0.78; 95% CI, 0.61 to 0.98; P = 0.03), representing a 4.1-month improvement in median OS (25.8 months in sipuleucel-T group vs. 21.7 months in placebo group).	[9]

Sr. no.	Drug name	Mechanism of action	Key trials (phase III)	Patient population	Trial characteristics	Outcomes with respect to primary end points	References
Side effects:		Side effects included chills, pyrexia, headache, myalgia, influenza-like illness, hypertension, and hyperhidrosis.					[89]
3	Denosumab	A human IgG2 recombinant monoclonal antibody that binds to RANKL, inhibiting its interaction with the receptor RANK, found abundantly on osteoclasts, consequently reducing bone resorption, osteoclast development, function, and survival [90]	NCT00321620 (phase III trial)	1904 patients with hormone-refractory prostate cancer	Denosumab + zoledronic acid placebo vs. zoledronic acid + denosumab placebo	Median OS in denosumab group was 20.7 months (95% CI 18.8–24.9) as compared to 17.1 months for zoledronic acid group (hazard ratio 0.82, 95% CI 0.71–0.95; P = 0.0002)	[91]
Side effects:		Side effects included hypocalcemia and osteonecrosis of the jaw.					[91]

Table 1.
Summary of key trials of current FDA-approved treatments for CRPC and mCRPC.

beginning of precision therapy in PCa. This has significantly advanced our knowledge of and ability to treat this illness.

The translation of precision medicine approaches to clinical practices for CRPC presents various challenges. A major limitation is obtaining tissue samples due to the procedures often being painful and invasive, especially in cases where tissue needs to be obtained from bone metastasis. Even after obtaining tissue samples, single biopsies are not enough to provide information on the entirety of the disease, as they cannot fully map out the genetic mutations and molecular heterogeneity across all metastatic sites, therefore leads to difficulty in the selection of effective therapies. Moreover, delays due to the processing of the sample or the analysis of sample data can make the information irrelevant for the treatment of patients with rapidly progressing CRPC.

In practice, precision medicine requires infrastructure and resources such as advanced sample processing tools, bioinformatics and data analysis tools, and specialized clinical expertise, which may not be available in many centers that patients visit for treatment. Additionally, though targeted treatments may be used, many cases develop resistance toward these therapies, hindering their effectiveness in completely treating the disease. Apart from clinical limitations, these resources are often expensive, restricting their widespread availability. Another limitation includes the patient's eligibility for testing, consent to undergo additional procedures and the privacy of their data, raising ethical concerns and hindering the practicality of the adoption of these approaches.

Though precision medicine holds great promise in CRPC treatment, these limitations underline the necessity of accessible technologies, resources, and further clinical evidence to be fully adopted in clinical settings.

7.2 Improving patient outcomes and quality of life

Lifelong ADT and use of ARSIs demonstrate an increase in fatigue, hot flashes, dizziness, and appetite loss, often being linked to various adverse effects on cardiovascular, sexual, muscular, and bone health, leading to low quality of life in patients with mCRPC. Comprehensive pain management through opioid analgesics, EBRT, and radionuclide therapy is essential in mitigating cancer-associated pain. Early integration of palliative care is shown to enhance quality of life by addressing cachexia, fatigue, and psychological distress [94].

8. Conclusion

Castrate resistant prostate cancer remains a significant clinical challenge due to its persistent progression despite ADT. Current risk prediction methods and chemotherapeutic, immunotherapeutic, and radiation-based therapeutic strategies targeting AR dependent and independent mechanisms have been shown to improve early detection rates, prognosis, and patient survival. However, challenges remain due to the development of resistance against existing treatments. Ongoing research continues to refine the clinical landscape, integrating novel therapeutic strategies to enhance efficiency and extend survival.

Acknowledgements

We express our gratitude to Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Tathawade, Pune for infrastructural assistance

for carrying out this work. Vishal Kumar Sahu acknowledges the Council of Scientific and Industrial Research (CSIR), New Delhi for CSIR-SRF fellowship (File No.: 09/1304(11487)/2021-EMR-I.

Funding

This work is supported by the Ramalingaswami Re-entry fellowship, Department of Biotechnology, Govt. of India to S. Sur [BT/RLF/Re-entry/47/2021] and Intramural Grants by Dr. D. Y. Patil Vidyapeeth (DPU), Pimpri, Pune, India to S. Basu [DPU/644-43/2021] and [DPU/1211 (3)/2024], and to A. Ranjan [DPU/1211 (2)/2024].

Conflict of interest

The authors declare no conflict of interest.

Author details

Soumya Basu*, Akshita Saravanan[†], Roshni Matekar[†], Samiksha Bujone, Saanvi Vishnoi, Vishal Kumar Sahu, Subhayan Sur and Amit Ranjan*
Cancer and Translational Research Centre, Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Pune, India

*Address all correspondence to: soumya.bs@gmail.com; soumya.basu@dpu.edu.in and amith.ranjan@dpu.edu.in

[†] These authors contributed equally.

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] Mattiuzzi C, Lippi G. Current cancer epidemiology. *Journal of Epidemiology and Global Health*. 2019;**9**(4):217-222. DOI: 10.2991/jegh.k.191008.001
- [2] 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. Epub 2025 Jan 16
- [3] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a Cancer Journal for Clinicians*. 2018;**68**(6):394-424. DOI: 10.3322/caac.21492. Epub 2018 Sep 12. Erratum in: *CA Cancer J Clin*. 2020; **70** (4):313. doi: 10.3322/caac.21609
- [4] Sathishkumar K, Chaturvedi M, Das P, Stephen S, Mathur P. Cancer incidence estimates for 2022 & projection for 2025: Result from National Cancer Registry Programme, India. *The Indian Journal of Medical Research*. 2022;**156**(4&5):598-607. DOI: 10.4103/ijmr.ijmr_1821_22
- [5] Leslie SW, Soon-Sutton TL, Skelton WP. Prostate cancer. In: *StatPearls [Internet]*. Treasure Island (FL): StatPearls Publishing; 2025. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK470550/#>
- [6] Bleyer A, Spreafico F, Barr R. Causation of increased prostate cancer in young men. *Oncoscience*. 2021;**8**:37-39. DOI: 10.18632/oncoscience.527
- [7] National Cancer Institute. Castrate-Resistant Prostate Cancer. n.d. Available from: <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/castrate-resistant-prostate-cancer> [Accessed: March 24, 2025]
- [8] Ozyigit G, Hurmuz P, Yuce D, Akyol F. Prognostic significance of castrate testosterone levels for patients with intermediate and high risk prostate cancer. *World Journal of Clinical Oncology*. 2019;**10**(8):283-292. DOI: 10.5306/wjco.v10.i8.283
- [9] Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *The New England Journal of Medicine*. 2010;**363**(5):411-422. DOI: 10.1056/NEJMoa1001294
- [10] Gater A, Abetz-Webb L, Battersby C, Parasuraman B, McIntosh S, Nathan F, et al. Pain in castration-resistant prostate cancer with bone metastases: A qualitative study. *Health and Quality of Life Outcomes*. 2011;**9**:88. DOI: 10.1186/1477-7525-9-88
- [11] Tilki D, Salami SS, Sharifi N. Understanding mechanisms of resistance in metastatic castration-resistant prostate cancer: The role of the androgen receptor. *European Urology Focus*. 2016;**2**(5):499-505. DOI: 10.1016/j.euf.2016.04.003
- [12] Tan ME, Li J, Xu HE, Melcher K, Yong E. Androgen receptor: Structure, role in prostate cancer and drug discovery. *Acta Pharmacologica Sinica*. 2014;**36**(1):3-23. DOI: 10.1038/aps.2014.18
- [13] Crona DJ, Whang YE. Androgen receptor-dependent and -independent mechanisms involved in prostate cancer therapy resistance. *Cancers (Basel)*. 2017;**9**(6):67. DOI: 10.3390/cancers9060067

- [14] Huang Y, Jiang X, Liang X, Jiang G. Molecular and cellular mechanisms of castration resistant prostate cancer. *Oncology Letters*. 2018;**15**(5):6063-6076. DOI: 10.3892/ol.2018.8123. Epub 2018 Feb 27
- [15] Vellky JE, Ricke WA. Development and prevalence of castration-resistant prostate cancer subtypes. *Neoplasia*. 2020;**22**(11):566-575. DOI: 10.1016/j.neo.2020.09.002
- [16] Crowley F, Sterpi M, Buckley C, Margetich L, Handa S, Dovey Z. A review of the pathophysiological mechanisms underlying castration-resistant prostate cancer. *Research and Reports in Urology*. 2021;**13**:457-472. DOI: 10.2147/RRU.S264722
- [17] Chandrasekar T, Yang JC, Gao AC, Evans CP. Mechanisms of resistance in castration-resistant prostate cancer (CRPC). *Translational Andrology and Urology*. 2015;**4**(3):365-380. DOI: 10.3978/j.issn.2223-4683.2015.05.02
- [18] Dutt SS, Gao AC. Molecular mechanisms of castration-resistant prostate cancer progression. *Future Oncology*. 2009;**5**(9):1403-1413. DOI: 10.2217/fon.09.117
- [19] Yang L, Lin C, Jin C, Yang JC, Tanasa B, Li W, et al. LncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. *Nature*. 2013;**500**(7464):598-602. DOI: 10.1038/nature12451
- [20] Liu R, Zou Z, Zhang Z, He H, Xi M, Liang Y, et al. Evaluation of glucocorticoid-related genes reveals GPD1 as a therapeutic target and regulator of sphingosine 1-phosphate metabolism in CRPC. *Cancer Letters*. 2024;**605**:217286. DOI: 10.1016/j.canlet.2024.217286
- [21] Yamada Y, Beltran H. Clinical and biological features of neuroendocrine prostate cancer. *Current Oncology Reports*. 2021;**23**(2):15. DOI: 10.1007/s11912-020-01003-9
- [22] Hoang DT, Iczkowski KA, Kilari D, See W, Nevalainen MT. Androgen receptor-dependent and -independent mechanisms driving prostate cancer progression: Opportunities for therapeutic targeting from multiple angles. *Oncotarget*. 2017;**8**:3724-3745. DOI: 10.18632/oncotarget.12554
- [23] Patel JC, Maughan BL, Agarwal AM, Batten JA, Zhang TY, Agarwal N. Emerging molecularly targeted therapies in castration-refractory prostate cancer. *Prostate Cancer*. 2013;**2013**:981684. DOI: 10.1155/2013/981684
- [24] Smith MR, Kabbinnavar F, Saad F, Hussain A, Gittelman MC, Bilhartz DL, et al. Natural history of rising serum prostate-specific antigen in men with castrate nonmetastatic prostate cancer. *Journal of Clinical Oncology*. 2005;**23**(13):2918-2925. DOI: 10.1200/JCO.2005.01.529
- [25] Widmark A, Klepp O, Solberg A, Damber JE, Angelsen A, Fransson P, et al. Endocrine treatment, with or without radiotherapy, in locally advanced prostate cancer (SPCG-7/SFUO-3): An open randomised phase III trial. *Lancet*. 2009;**373**(9660):301-308. DOI: 10.1016/S0140-6736(08)61815-2
- [26] Chen S, Jian T, Chi C, Liang Y, Liang X, Yu Y, et al. Machine learning-based models enhance the prediction of prostate cancer. *Frontiers in Oncology*. 2022;**12**:941349. DOI: 10.3389/fonc.2022.941349
- [27] López-Campos F, Conde-Moreno A, Barrado Los Arcos M, Gómez-Caamaño A, García-Gómez R, Hervás Morón A.

Treatment landscape of nonmetastatic castration-resistant prostate cancer: A window of opportunity. *Journal of Personalized Medicine*. 2021;**11**(11):1190. DOI: 10.3390/jpm11111190

[28] Zheng H, Chen J, Qiu W, Lin S, Chen Y, Liang G, et al. Safety and efficacy of first-line treatments for chemotherapy-naïve metastatic castration-resistant prostate cancer: A systematic review and indirect comparison. *BioMed Research International*. 2017;**2017**:3941217. DOI: 10.1155/2017/3941217

[29] Liu Y, Yao ZX, Papadopoulos V. Cytochrome P450 17alpha hydroxylase/17,20 lyase (CYP17) function in cholesterol biosynthesis: Identification of squalene monooxygenase (epoxidase) activity associated with CYP17 in Leydig cells. *Molecular Endocrinology*. 2005;**19**(7):1918-1931. DOI: 10.1210/me.2004-0271. Epub 2005 Mar 10

[30] Rice MA, Malhotra SV, Stoyanova T. Second-generation antiandrogens: From discovery to standard of care in castration resistant prostate cancer. *Frontiers in Oncology*. 2019;**9**:801. DOI: 10.3389/fonc.2019.00801

[31] Tresnanda RI, Pramod SV, Safriadi F. Ketoconazole for the treatment of docetaxel-naïve metastatic castration-resistant prostate cancer (mCRPC): A systematic review. *Asian Pacific Journal of Cancer Prevention*. 2021;**22**(10):3101-3107. DOI: 10.31557/APJCP.2021.22.10.3101

[32] Van Hook K, Huang T, Alumkal JJ. Orteronel for the treatment of prostate cancer. *Future Oncology*. 2014;**10**(5):803-811. DOI: 10.2217/fon.14.35

[33] Takeda Pharmaceutical Company Limited. Takeda Announces Termination

of Orteronel (TAK-700) Development for Prostate Cancer in Japan, U.S.A. and Europe. 2014. Available from: <https://www.takeda.com/newsroom/newsreleases/2014/takeda-announces-termination-of-orteronel-tak-700-development-for-prostate-cancer-in-japan-usa-and-europe/> [Accessed: March 24, 2025]

[34] McKay RR, Werner L, Fiorillo M, Roberts J, Heath EI, Bubley GJ, et al. Efficacy of therapies after galeterone in patients with castration-resistant prostate cancer. *Clinical Genitourinary Cancer*. 2017;**15**(4):463-471. DOI: 10.1016/j.clgc.2016.10.006. Epub 2016 Oct 27

[35] Kuzminac IZ, Nikolić AR, Savić MP, Ajduković JJ. Abiraterone and galeterone, powerful tools against prostate cancer: Present and perspective. *Pharmaceutics*. 2024;**16**(11):1401. DOI: 10.3390/pharmaceutics16111401

[36] U.S. Food and Drug Administration. FDA Approves Abiraterone Acetate in Combination with Prednisone for High-Risk Metastatic Castration-Sensitive Prostate Cancer. 2024. Available from: <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-abiraterone-acetate-combination-prednisone-high-risk-metastatic-castration-sensitive> [Accessed: March 24, 2025]

[37] Stein MN, Patel N, Bershadskiy A, Sokoloff A, Singer EA. Androgen synthesis inhibitors in the treatment of castration-resistant prostate cancer. *Asian Journal of Andrology*. 2014;**16**(3):387-400. DOI: 10.4103/1008-682X.129133

[38] Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY, et al. Abiraterone plus prednisone in metastatic,

castration-sensitive prostate cancer. The New England Journal of Medicine. 2017;**377**(4):352-360. DOI: 10.1056/NEJMoa1704174

[39] Rathkopf D, Scher HI. Androgen receptor antagonists in castration-resistant prostate cancer. Cancer Journal. 2013;**19**(1):43-49. DOI: 10.1097/PPO.0b013e318282635a

[40] Ning YM, Brave M, Maher VE, Zhang L, Tang S, Sridhara R, et al. U.S. Food and Drug Administration approval summary: Enzalutamide for the treatment of patients with chemotherapy-naïve metastatic castration-resistant prostate cancer. The Oncologist. 2015;**20**(8):960-966. DOI: 10.1634/theoncologist.2015-0166. Epub 2015 Jun 12

[41] Freedland SJ, de Almeida LM, De Giorgi U, Saad F, Yu EY, Ross RW, et al. Improved outcomes with enzalutamide in biochemically recurrent prostate cancer. The New England Journal of Medicine. 2023;**389**(16):1453-1465. DOI: 10.1056/NEJMoa2303974

[42] U.S. Food and Drug Administration. FDA Approves Apalutamide for Non-Metastatic Castration-Resistant Prostate Cancer. 2018. Available from: <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-apalutamide-non-metastatic-castration-resistant-prostate-cancer> [Accessed: March 24, 2025]

[43] U.S. Food and Drug Administration. FDA Approves Darolutamide for Non-Metastatic Castration-Resistant Prostate Cancer. 2019. Available from: <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-darolutamide-non-metastatic-castration-resistant-prostate-cancer> [Accessed: March 24, 2025]

[44] Laccetti AL et al. Phase 1/2 study of EPI-7386 in combination with enzalutamide compared with enzalutamide alone in subjects with metastatic castration-resistant prostate cancer (mCRPC). Journal of Clinical Oncology. 2023;**41**(Suppl. 6):179. DOI: 10.1200/JCO.2023.41.6_suppl.179

[45] Zhao S, Yu EY. Castrate-resistant prostate cancer: Postdocetaxel management. Current Opinion in Urology. 2013;**23**(3):201-207. DOI: 10.1097/MOU.0b013e32835e2253

[46] Santos-Amaral TM, Macedo D, Fernandes I, Costa L. Castration-resistant prostate cancer: Mechanisms, targets, and treatment. Prostate Cancer. 2012;**2012**:327253. DOI: 10.1155/2012/327253

[47] Teply BA, Hauke RJ. Chemotherapy options in castration-resistant prostate cancer. Indian Journal of Urology. 2016;**32**(4):262-270. DOI: 10.4103/0970-1591.191239

[48] Bilusic M, Dahut WL. Cabazitaxel: A new drug for metastatic prostate cancer. Asian Journal of Andrology. 2011;**13**(2):185-186. DOI: 10.1038/aja.2010.161. Epub 2010 Dec 13

[49] Den RB, George D, Pieczonka C, McNamara M. Ra-223 treatment for bone metastases in castrate-resistant prostate cancer: Practical management issues for patient selection. American Journal of Clinical Oncology. 2019;**42**(4):399-406. DOI: 10.1097/COC.0000000000000528

[50] Shulman RM, Meyer JE, Li T, Howell KJ. External beam radiation therapy (EBRT) for asymptomatic bone metastases in patients with solid tumors reduces the risk of skeletal-related events (SREs). Annals of Palliative Medicine. 2019;**8**(2):159-167. DOI: 10.21037/apm.2018.10.04. Epub 2018 Nov 6

- [51] Rose JN, Crook JM. The role of radiation therapy in the treatment of metastatic castrate-resistant prostate cancer. *Therapeutic Advances in Urology*. 2015;7(3):135-145. DOI: 10.1177/1756287215576647
- [52] De Felice F, Piccioli A, Musio D, Tombolini V. The role of radiation therapy in bone metastases management. *Oncotarget*. 2017;8(15):25691-25699. DOI: 10.18632/oncotarget.14823
- [53] Cai M, Song X, Li X, Chen M, Guo J, Yang D, et al. Current therapy and drug resistance in metastatic castration-resistant prostate cancer. *Drug Resistance Updates*. 2023;68:100962. DOI: 10.1016/j.drug.2023.100962
- [54] Staniszevska M, Iking J, Lückerrath K, Hadaschik B, Herrmann K, Ferdinandus J, et al. Drug and molecular radiotherapy combinations for metastatic castration-resistant prostate cancer. *Nuclear Medicine and Biology*. 2021;96-97:101-111. DOI: 10.1016/j.nucmedbio.2021.03.009
- [55] Kluetz PG, Pierce W, Maher VE, Zhang H, Tang S, Song P, et al. Radium Ra 223 dichloride injection: U.S. Food and Drug Administration drug approval summary. *Clinical Cancer Research*. 2014;20(1):9-14. DOI: 10.1158/1078-0432.CCR-13-2665. Epub 2013 Nov 4
- [56] Lanka SM, Zorko NA, Antonarakis ES, Barata PC. Metastatic castration-resistant prostate cancer, immune checkpoint inhibitors, and beyond. *Current Oncology*. 2023;30(4):4246-4256. DOI: 10.3390/curroncol30040323
- [57] Cheever MA, Higano CS. PROVENGE (Sipuleucel-T) in prostate cancer: The first FDA-approved therapeutic cancer vaccine. *Clinical Cancer Research*. 2011;17(11):3520-3526. DOI: 10.1158/1078-0432.CCR-10-3126. Epub 2011 Apr 6
- [58] Miller DR, Ingersoll MA, Teply BA, et al. Combination treatment options for castration-resistant prostate cancer. In: Bott SRJ, Ng KL, editors. *Prostate Cancer*. Brisbane (AU): Exon Publications; 2021. Chapter 5. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK571328/>. DOI: 10.36255/exonpublications.prostatecancer.combinationtreatment.2021
- [59] U.S. Food and Drug Administration. Full Prescribing Information for Sipuleucel-T (Provenge). 2013. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125320s094lbl.pdf [Accessed: March 24, 2025]
- [60] Carrese B, Sanità G, Lamberti A. Nanoparticles design for theranostic approach in cancer disease. *Cancers (Basel)*. 2022;14(19):4654. DOI: 10.3390/cancers14194654
- [61] Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. *The New England Journal of Medicine*. 2015;373(8):737-746. DOI: 10.1056/NEJMoa1503747. Epub 2015 Aug 5
- [62] Knudsen KE, Scher HI. Starving the addiction: New opportunities for durable suppression of AR signalling in prostate cancer. *Clinical Cancer Research*. 2009;15(15):4792-4798. DOI: 10.1158/1078-0432.CCR-08-2660. Epub 2009 Jul 28
- [63] Miyahira AK, Soule HR. The history of prostate-specific membrane antigen as a theranostic target in prostate cancer: The cornerstone role of the prostate cancer foundation. *Journal of Nuclear Medicine*. 2022;63(3):331-338.

DOI: 10.2967/jnumed.121.262997.
Epub 2021 Oct 21

[64] Maurer T, Eiber M, Schwaiger M, Gschwend JE. Current use of PSMA-PET in prostate cancer management. *Nature Reviews. Urology*. 2016;**13**(4):226-235. DOI: 10.1038/nrurol.2016.26. Epub 2016 Feb 23

[65] Jadvar H. PSMA PET in prostate cancer. *Journal of Nuclear Medicine*. 2015;**56**(8):1131-1132. DOI: 10.2967/jnumed.115.157339. Epub 2015 May 14

[66] Silver DA, Pellicer I, Fair WR, Heston WD, Cordon-Cardo C. Prostate-specific membrane antigen expression in normal and malignant human tissues. *Clinical Cancer Research*. 1997;**3**(1):81-85

[67] Perera M, Papa N, Christidis D, Wetherell D, Hofman MS, Murphy DG, et al. Sensitivity, specificity, and predictors of positive 68Ga-prostate-specific membrane antigen positron emission tomography in advanced prostate cancer: A systematic review and meta-analysis. *European Urology*. 2016;**70**(6):926-937. DOI: 10.1016/j.eururo.2016.06.021. Epub 2016 Jun 28

[68] Perrone E, Giordano A, Calcagni ML, Leccisotti L, Moretti R, Eismant A, et al. Long-term safety and survival outcomes of [225Ac]Ac-PSMA (prostate-specific membrane antigen) and [225Ac]Ac-/ [177Lu]Lu-PSMA (TANDEM) radioligand therapy (PRLT) in metastatic castration-resistant prostate cancer. *Cancers (Basel)*. 2025;**17**(3):405. DOI: 10.3390/cancers17030405

[69] Staniszewska M, Fragoso Costa P, Eiber M, Klose JM, Wosniack J, Reis H, et al. Enzalutamide enhances PSMA expression of PSMA-low prostate cancer. *International Journal of Molecular Sciences*. 2021;**22**(14):7431. DOI: 10.3390/ijms22147431

[70] Powers E, Karachaliou GS, Kao C, Harrison MR, Hoimes CJ, George DJ, et al. Novel therapies are changing treatment paradigms in metastatic prostate cancer. *Journal of Hematology & Oncology*. 2020;**13**(1):144. DOI: 10.1186/s13045-020-00978-z

[71] Rehman Y, Rosenberg JE. Abiraterone acetate: Oral androgen biosynthesis inhibitor for treatment of castration-resistant prostate cancer. *Drug Design, Development and Therapy*. 2012;**6**:13-18. DOI: 10.2147/DDDT.S15850. Epub 2012

[72] de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. *The New England Journal of Medicine*. 2011;**364**(21):1995-2005. DOI: 10.1056/NEJMoa101461

[73] Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. *The New England Journal of Medicine*. 2013;**368**(2):138-148. DOI: 10.1056/NEJMoa1209096

[74] Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *The New England Journal of Medicine*. 2012;**367**(13):1187-1197. DOI: 10.1056/NEJMoa1207506

[75] Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. *The New England Journal of Medicine*. 2014;**371**(5):424-433. DOI: 10.1056/NEJMoa1405095

[76] Sternberg CN, Fizazi K, Saad F, Shore ND, De Giorgi U, Penson DF, et al. Enzalutamide and survival in

nonmetastatic, castration-resistant prostate cancer. *The New England Journal of Medicine*. 2020;**382**(23):2197-2206. DOI: 10.1056/NEJMoa2003892

[77] Bhole RP, Karche PM, Gurav SS, Chikhale RV. Breaking through resistance in mCRPC: Enzalutamide analogues as effective anticancer agents for enhanced male survival. *Results in Chemistry*. 2023;**6**:101143. DOI: 10.1016/j.rechem.2023.101143

[78] Smith MR, Saad F, Chowdhury S, Oudard S, Stuchbery G, Hadaschik BA, et al. Apalutamide treatment and metastasis-free survival in prostate cancer. *The New England Journal of Medicine*. 2018;**378**(15):1408-1418. DOI: 10.1056/NEJMoa1715546

[79] Bögemann M, Facchini G, Bauernhofer T, Cathomas R, Xylinas E, Tombal B. Role of apalutamide in the treatment landscape for patients with advanced prostate cancer: An expert opinion statement of European clinical practice. *Irish Journal of Medical Science*. 2023;**192**(6):2643. DOI: 10.1007/s11845-023-03303-y

[80] Fizazi K, Shore N, Tammela TL, Ulys A, Vjaters E, Polyakov S, et al. Darolutamide in nonmetastatic, castration-resistant prostate cancer. *The New England Journal of Medicine*. 2019;**380**(13):1235-1246. DOI: 10.1056/NEJMoa1815671

[81] Yang C, Cha T, Chang Y, Huang S, Lin J, Wang S, et al. Darolutamide for non-metastatic castration-resistant prostate cancer: Efficacy, safety, and clinical perspectives of use. *Journal of the Formosan Medical Association*. 2023;**122**(4):299-308. DOI: 10.1016/j.jfma.2022.12.008

[82] Armstrong CM, Gao AC. Drug resistance in castration resistant prostate

cancer: Resistance mechanisms and emerging treatment strategies. *American Journal of Clinical and Experimental Urology*. 2015;**3**(2):64-76

[83] Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, et al. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. *The New England Journal of Medicine*. 2004;**351**(15):1502-1512. DOI: 10.1056/NEJMoa040720

[84] de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic prostate cancer progressing after docetaxel. *Lancet*. 2010;**376**(9747):1147-1154. DOI: 10.1016/S0140-6736(10)61389-X

[85] de Wit R, de Bono J, Sternberg CN, Fizazi K, Tombal B, Wülfing C, et al. Cabazitaxel versus abiraterone or enzalutamide in metastatic prostate cancer. *The New England Journal of Medicine*. 2019;**381**(26):2506-2518. DOI: 10.1056/NEJMoa1911206

[86] Abidi A. Cabazitaxel: A novel taxane for metastatic castration-resistant prostate cancer—Current implications and future prospects. *Journal of Pharmacology and Pharmacotherapeutics*. 2013;**4**(4):230-232. DOI: 10.4103/0976-500X.119704

[87] Petrylak DP. Practical guide to the use of chemotherapy in castration resistant prostate cancer. *The Canadian Journal of Urology*. 2014;**21** (2 Suppl. 1):77-83

[88] Antonarakis ES, Piulats JM, Gross-Goupil M, Goh J, Ojamaa K, Hoimes CJ, et al. Pembrolizumab for treatment-refractory metastatic castration-resistant prostate cancer: Multicohort, open-label phase II

KEYNOTE-199 study. *Journal of Clinical Oncology*. 2020;**38**(5):395-405.
DOI: 10.1200/JCO.19.01638

[89] Graff JN, Chamberlain ED.
Sipuleucel-T in the treatment of prostate cancer: An evidence-based review of its place in therapy. *Core Evidence*. 2015;**10**:1-10. DOI: 10.2147/CE.S54712

[90] Mollica V, Nuvola G, Tassinari E, Nigro MC, Marchetti A, Rosellini M, et al. Bone targeting agents in patients with prostate cancer: General toxicities and osteonecrosis of the jaw. *Current Oncology*. 2022;**29**(3):1709-1722.
DOI: 10.3390/curroncol29030142

[91] Fizazi K, Carducci M, Smith M, Damião R, Brown J, Karsh L, et al. Denosumab versus zoledronic acid for treatment of bone metastases in men with castration-resistant prostate cancer: A randomised, double-blind study. *Lancet*. 2011;**377**(9768):813-822. DOI: 10.1016/S0140-6736(10)62344-6. Epub 2011 Feb 25

[92] Kulasegaran T, Oliveira N. Metastatic castration-resistant prostate cancer: Advances in treatment and symptom management. *Current Treatment Options in Oncology*. 2024;**25**(7):914-931. DOI: 10.1007/s11864-024-01215-2. Epub 2024 Jun 24

[93] Fenton SE et al. Advancing prostate cancer care: Treatment approaches to precision medicine, biomarker innovations, and equitable access. *American Society of Clinical Oncology Educational Book*. 2024;**44**:e433138.
DOI: 10.1200/EDBK_433138

[94] Joly F, Oudard S, Fizazi K, Tubach F, Jove J, Lacueille C, et al. Quality of life and pain during treatment of metastatic castration-resistant prostate cancer with cabazitaxel in routine clinical practice. *Clinical Genitourinary Cancer*. 2020;**18**(5):e510-e516. DOI: 10.1016/j.clgc.2020.02.003

Production of Human Prostate Adenocarcinoma Tumoroids for Antitumor Testing

Patrícia Falcão and Carlos Roberto Jorge Soares

Abstract

This chapter proposes the use of three-dimensional (3D) cell culture strategies that, by emulating the physiological characteristics of tissues composed of mammalian cells, allow for the large-scale production of spheroids from human prostate adenocarcinoma tumor cells. It is worth noting that the extended use of 3D cultures has also enabled the investigation of biomolecular mechanisms in cell lines from other types of tumors that are resistant to the conventional treatments, as they can more accurately represent tumor physiology (tumoroids) in an *in vitro* setting. The 3D culture has proven effective in investigating biomolecular mechanisms of highly aggressive tumor cell lines. Preliminary results indicate that key signaling pathways involved in the maintenance of clonogenesis can be successfully explored using three-dimensional cultures.

Keywords: prostate tumor, human prostate epithelial cell line (RWPE-1), LNCaP cell line, signaling pathways, cytokines

1. Introduction

This chapter, entitled “*Production of Human Prostate Adenocarcinoma Tumoroids for Antitumor Testing*,” briefly addresses the search for study models supported by the representativeness of various tumor types *in vivo*, allowing the investigation of pathophysiological aspects and mechanisms that may be directly or indirectly involved in the prognosis of the disease. Studies of such scope are justified by the epidemiology of cancer as a serious disease.

Cancer is the leading public health problem worldwide and currently ranks among the four main causes of premature death in most countries (INCA 2023). The increasing incidence and mortality of cancer globally justifies the pursuit of knowledge regarding tumor behavior in response to the therapeutic approaches currently in use, as well as the investigation of its direct impact on disease prognosis. The search for new therapeutic targets, considering the grim prognosis and the behavior of aggressive tumor lineages, must converge toward the use of models that most

faithfully resemble a tumor *in vivo*, within the context of its abnormal metabolism. This abnormal metabolism appears to play an important role in the understanding of tumorigenesis, metastasis, and resistance to drugs and radiotherapy.

Considering prostate cancer as the second most common type of cancer among men worldwide, after lung cancer, more than 65,000 new cases per year are expected. Its incidence (29.2%) is more than three times higher than that of the second most common group, colon and rectal cancer (9.1% in men). Malignant prostate neoplasms, together with breast cancer, are the most frequent cancer types in Brazil. For this reason, prostate cancer research is of great relevance in the field of Human Health [1].

With regard to prostate tumors and prostate adenocarcinomas, it is worth briefly describing the anatomy of the prostate. It is a pyramidal gland weighing about 15–20 g when healthy, located below the male bladder and surrounding the urethra. Its major function is to produce prostatic fluid, the main component of ejaculate. Histologically, the organ is composed of two matrices: smooth muscle and glandular [2]. The glandular matrix is characterized by acinar ductal structures, separated from the muscle matrix by a pseudostratified epithelium formed by cuboidal basal cells and columnar secretory cells polarized toward the interior of the acini. These structures are delimited by their own basal lamina and populate the entire parenchyma of the organ [3].

It is worth mentioning that prostate tumors are more frequent in the peripheral zone (about 75%), followed by the transitional zone (20%) and the central zone (5%) [4]. Prostate tumors may arise in different regions of the organ parenchyma. According to their origin, they are classified as squamous cell carcinoma, basal adenocarcinoma, neuroendocrine carcinoma, luminal adenocarcinoma, and small-cell carcinoma [5]. Adenocarcinomas, in turn, are highly diverse, developing in several morphological subtypes [6]. Standard tumor pathology is characterized by the fusion of normal acinar structures with a drastic reduction in lumen space [7]. Epithelial cells are diffusely distributed throughout the organ parenchyma, compressing the muscle matrix and predominating over it [8].

Regarding prostate cancer (PCa) therapy, androgen deprivation therapy is reported to be highly effective in treating patients with advanced PCa, as androgen signaling is essential for PCa growth and the maintenance of its anti-apoptotic capacity [9]. However, despite the initial efficacy of this therapy, patients eventually develop resistance, leading to recurrence and/or progression to castration-resistant prostate cancer (CRPC) [10]. Although new androgen receptor (AR)-targeted therapies, such as enzalutamide (ENZ) and abiraterone [11, 12], as well as the chemotherapeutic agent cabazitaxel [13], have been approved and used in the treatment of CRPC patients, these drugs are associated with limited efficacy. Moreover, while most CRPC tumors exhibit AR-dependent growth through AR-activating mutations, amplification, or splice variants, up to 10–20% of CRPC tumors lose their AR dependency to escape the AR-targeted therapy. One manifestation is the transformation of AR-positive adenocarcinomas into AR-negative small-cell neuroendocrine prostate carcinomas (NEPCs). Treatment-related NEPCs account for approximately 25% of the nearly 34,000 lethal PCa cases diagnosed annually worldwide [14].

As described in the most recent studies, understanding the mechanisms associated with treatment response in PCa is complex in terms of tumor behavior. Thus, preclinical PCa models have been developed to elucidate the complex mechanisms underlying treatment resistance. Three PCa cell lines developed in the 1970s and 1980s, namely LNCaP [15], DU145 [16], and PC-3 [17], remain the most widely used

PCa cell lines in published studies [18]. Established cell lines are selected from specific tumor subsets that grow under *in vitro* culture conditions. This selection process produces cancer cell lines that do not represent the diversity of human tumors [19]. Furthermore, PCa cell lines cultured exclusively in monolayers lack the heterogeneity observed in patient-derived PCa cells [20]. By activating AR mutations, amplification, or splice variants, up to 10–20% of CRPC tumors lose their AR dependency to escape AR-targeted therapy [21]. One manifestation is the transformation of AR-positive adenocarcinomas into AR-negative small-cell neuroendocrine prostate carcinomas (NEPCs). Treatment-related NEPCs account for approximately 25% of nearly 34,000 lethal disease cases.

In vitro culture techniques for producing prostate tumor cell spheroids represent a reproducible model capable of providing sufficient experimental material for large-scale tumor physiology research, using disruptive three-dimensional cell culture technologies. *In vitro* cell cultures provide the minimal biological and physiological configurations necessary for studies of various tissues. Adequate supply of nutrients and gases enables cell lines to reproduce, allowing their use in sufficient quantities as test systems for studies in different fields of knowledge. Conventional protocols are based on the so-called monolayer culture, in which cells grow attached to glass or plastic surfaces, arranged laterally to one another. Over the course of several days, cells cover the culture surface, forming a structure similar to a “carpet.” Among the most frequent applications of cell cultures are those dedicated to studying tumor physiology *in vitro*, usually aimed at investigating tumor susceptibility to antitumor therapeutics [22–26]. Although these systems have been widely used for about 50 years [27, 28], current scientific evidence demonstrates that the two-dimensional arrangement prevents cells from expressing their characteristics analogously to those found in the organism [29]. For such lineages to behave more closely to those observed *in vivo*, models that allow three-dimensional cell interactions have been proposed [30], thus favoring production and a certain degree of encapsulation, promoting interaction with extracellular matrix proteins [31–33].

Tumoroid production techniques aid in investigating the cellular machinery involved in the promotion of clonogenesis and tumor differentiation through signaling pathways that favor tumor viability and aggressive potential [34]. Tumoroids are considered multicellular structures composed of various cell populations and function as *in vitro* tumor organs [35, 36]. The term has also been used to designate structures derived from tumor biopsies from patients under treatment and represents a promising specialized medicine technique [37–41].

In this regard, the study conceived within the project was directed toward the production of a 3D prostate adenocarcinoma cell culture model containing both tumor and non-tumor cells, namely: human prostate adenocarcinoma cell line (LNCaP) and human prostate epithelial cell line (RWPE-1), with minimal chemical supplementation. The development of studies of this magnitude is relevant for generating human prostate tumoroids, including the addition of fibroblasts and endothelial cells that, once established, could assume the roles of CAFs and tumor endothelium. In theory, this system is based on intrinsic stimulation, reducing the importance of supplements while structuring physical support for tissues through extracellular matrix production. Furthermore, the use of bioprinting techniques could assist in organizing tumoroids which, although larger in size (surpassing the millimeter scale), could contain multiple cellular structures and thus more faithfully emulate the tumor organ, making them suitable for drug testing in the near future.

2. Methodology

In the first stage of this study, a standardized three-dimensional (3D) culture model was established for the formation of free-floating tumouroids in culture plates pre-treated with Pluronic® F-127. This coating prevented cell adhesion to the plate surface and enabled the formation of free spheroids that were detected in multiple fields within the culture wells. This approach offers significant advantages, as *in vitro* spheroid formation makes it possible to investigate cellular interactions within a system that mimics the tumor microenvironment, while also allowing the detection of biomolecular profiles under different experimental conditions.

Cell cultures were established using LNCaP cells (prostate lymph node carcinoma) and RWPE-1 cells (human prostate epithelial cells). Both cell lines were maintained in 25-cm² culture flasks containing RPMI 1640 medium (Sigma-Aldrich), supplemented with 10% (v/v) foetal bovine serum (Cultlab) and 1% (v/v) antibiotic solution (10,000 IU/mL penicillin, 10 mg/mL streptomycin; Gibco). Cultures were incubated at 37°C in a humidified atmosphere with 5% CO₂. Once cells reached 60–70% confluence, they were detached using 0.05% trypsin solution. For spheroid production, LNCaP and RWPE-1 cells were trypsinised, counted, and seeded at a density of 1×10^5 cells per well in 24-well plates previously coated with Pluronic® F-127 (0.5 g/mL in 2-propanol). Spheroid formation was observed after 3 days.

The pre-treatment of plates involved the addition of 1000 µL of Pluronic® F-127 solution to each well of 24-well culture plates, followed by incubation at room temperature for 24 hours under laminar flow conditions. After this period, the liquid was removed by suction, and the plates were dried under UV irradiation for 30 minutes to eliminate any risk of contamination.

Hydrogel preparation was performed using hydrolysed cellulose at a concentration of 0.75%, which was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and the pellet was resuspended in sodium alginate hydrogel at 2.5% in phosphate-buffered saline (PBS). For cell incorporation, LNCaP and RWPE-1 cells were resuspended after trypsinisation and counted at a density of 2.5×10^5 cells per well, forming a final solution of 500 µL. A volume of 100 µL was pipetted into wells of both 24- and 96-well plates and covered with 50 µL of sterile 2% calcium chloride solution to crosslink the alginate. After 5 minutes of incubation at 37°C, the solution was removed and replaced with fresh culture medium for further incubation.

For biofabrication, the Tinkercad software was used to design a prostate prototype, which was then processed with Slicer3D for slicing and programming. The prototype was combined at a 1:1 ratio with hydrogels containing 10% alginate, 0.75% processed cellulose, and 2×10^6 cells/mL. This mixture was used for the biofabrication of constructs that were subsequently crosslinked in 100 mM calcium chloride solution for 10 minutes. After removal of the solution, constructs were covered with culture medium and transferred to the incubator.

Different fluorochromes were used for 3D assays to evaluate cell viability and proliferation. SYTOX Green (Life Technologies, Carlsbad, CA) is a nucleic acid dye that penetrates cells with compromised membranes, binding to DNA and emitting intense fluorescence upon excitation, thus allowing visualization and quantification of dead cells. Hoechst 33342, a fluorescent dye that binds preferentially to adenine-thymine-rich regions of DNA, was employed to stain nuclei. Although not specific for viable cells, Hoechst was used in combination with SYTOX to differentiate between live and dead populations: Live cells stained only with Hoechst, while dead cells incorporated both dyes. Additionally, a far-red dye, which emits fluorescence in the near-infrared

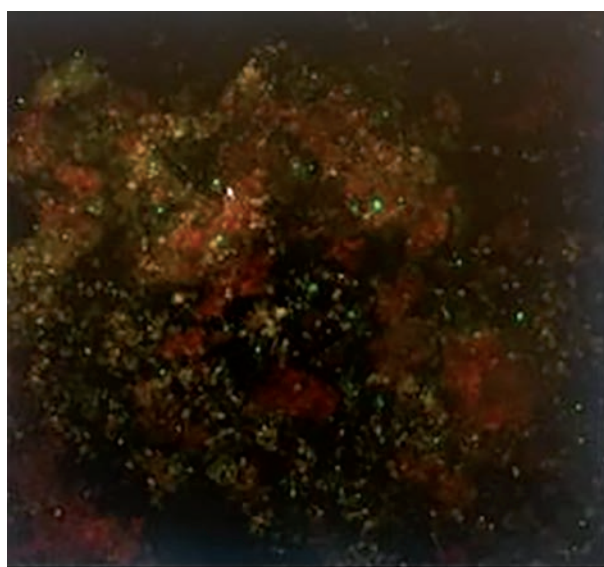


Figure 1.
 Representation of fluorochrome labelling detected during image acquisition by the INCell Analyzer 2500 HS.

spectrum (650–750 nm) upon excitation at ~633–647 nm, was employed for cell proliferation and cell cycle studies. This marker enabled the discrimination of cells at different phases of the cycle or following incorporation of fluorescent nucleotides (**Figure 1**).

High-resolution imaging and quantitative analysis were performed using the INCell Analyzer 2500 HS (Cytiva Biosciences). This platform allows high-content screening of large compound libraries, enabling the identification of substances with specific biological effects on cells. In the context of tumouroid production, it enabled live-cell imaging, time-lapse analysis, and evaluation of dynamic processes such as cell division, migration, and response to treatments. The integration of flow cytometry with imaging capabilities allowed detailed investigation of cell morphology, protein expression, and subcellular localization. This combination of techniques proved particularly useful for studying cancer biology, including the response of tumor cells to therapeutic interventions and the analysis of intracellular signaling pathways (**Figure 2**).

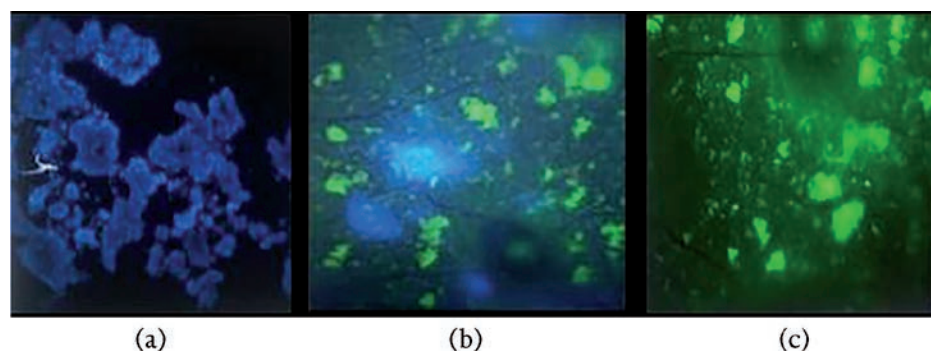


Figure 2.
 LNCaP cell spheroids stained with Hoechst-3342 and Sytox Green: (A) viable cells at 24 h; (B) viable and non-viable cells at 72 h; (C) non-viable cells after 8 days.

3. Discussion of the results of the 3D model and bioprinting

Tumor cell lines are the fundamental pillars of oncological research, offering unique advantages: They proliferate almost indefinitely and enable large-scale screening of new drugs [31]. From the classical prostate cancer cell lines—DU145, PC-3, and LNCaP—several sublines have been developed, each with distinct features, thereby broadening the range of tools available for investigating disease mechanisms and testing innovative therapeutic strategies. This constantly dividing cellular universe opens doors to discoveries that may transform the future of cancer treatment (**Figure 3**).

In this study, LNCaP cells were employed for the formation of tumor spheroids in antitumor assays. This cell line, isolated in 1977 by Horoszewicz and colleagues from a lymph node metastasis of a patient with prostate carcinoma [18], is widely recognized as a research model. Its principal morphological characteristics are adherence and epithelial morphology. From a genetic perspective, notable alterations include the T877A mutation in the androgen receptor (AR) gene, which allows the receptor to respond not only to androgens but also to other steroids and antiandrogens. Being androgen-sensitive, LNCaP proliferation is stimulated by testosterone and dihydrotestosterone (DHT), which makes them essential for studies concerning AR biology and the effects of androgen deprivation—a classical therapeutic strategy for advanced prostate cancer. Furthermore, these cells produce prostate-specific antigen (PSA), a central clinical biomarker in disease monitoring [10]. The line is also extensively used to investigate mechanisms of castration resistance, a condition that defines castration-resistant prostate cancer (CRPC) [11, 12].

The use of LNCaP cells in tumor spheroid formation proved to be a highly effective strategy to more faithfully replicate the cellular interactions that occur within the tumor microenvironment. This advancement is particularly noteworthy, as it provides an experimental setting closer to biological reality, allowing for deeper understanding of the mechanisms underpinning prostate cancer progression and responses to various therapeutic approaches.

The observation, within only 3 days of culture on plates treated with Pluronic® F-127, of the spontaneous organization of cells into spheroids demonstrates the dynamism and intrinsic complexity of this model. Images obtained using the INCell Analyzer 2500 HS (Cytiva Biosciences) reveal not only cellular migration and interaction but also the capacity of this system to provide information that transcends the traditional two-dimensional model. Through the integration of Hoechst-33,342 and SYTOX staining, it became possible to distinguish viable cells within the spheroids, thereby reinforcing the applicability of the model for functional and pharmacological analyses.

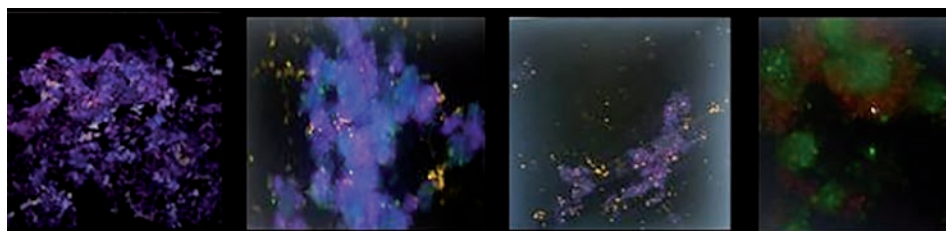


Figure 3.
Time course at 24, 48, and 72 h after tumor spheroid formation (LNCaP and RWPE-1) in 3D culture stained with Hoechst-33,342, Sytox Green, and Far-red.

It was confirmed that prolactin (hPRL) plays a relevant role in 3D cultures involving LNCaP cells, influencing their differentiation, proliferation, and clonogenicity over culture periods of 24, 48, and 72 hours. A clear difference was observed between 3D cultures treated with hPRL compared with those containing LNCaP cells alone. This response was further accentuated by the presence of RWPE-1 (human prostate epithelial cells) and peripheral blood mononuclear cells (PBMCs), resulting in increased cell viability (**Figure 4**).

Even cells exposed to 2 Gy of gamma radiation (Cobalt-60) maintained a response comparable to that of hPRL-treated cells, exhibiting tumor proliferation and reduced mortality. Moreover, RWPE-1 epithelial cells, as previously demonstrated in *in vivo* studies, surrounded the spheroid during its formation, even in the presence of PBMCs—particularly when hPRL was present. PBMCs migrated into the spheroid within the first 4 hours of incubation, concentrating mainly in the tumor region, which suggests the preferential recognition of the environment (**Figure 5**).

Preliminary findings further included the investigation of spheroid viability formed by LNCaP and RWPE-1 cells within a bioprinted prostate construct, developed in the Master's dissertation project of Da Silva (2024). This process, based on hydrolysed cellulose and sodium alginate as described in the methodology, is protected, as it will be the subject of a future patent application by the researcher (**Figure 6**) [42].

In continuation, preliminary data also explored the viability of spheroids within a bioprinted prostate model developed from nanocellulose and sodium alginate (Da Silva GD, Master's dissertation project, 2024) [42]. This innovative construct proved capable of maintaining, for 14 days, the viability of LNCaP cells in both the lumen and the internal periphery, as well as RWPE-1 cells incorporated into the hydrogel matrix. Fluorescence characterization (Hoechst-33,342, SYTOX Green, DID, and DIO) confirmed cellular interaction and survival (**Figure 6**). These results highlight the potential applications not only in tissue engineering but also in employing the system as an alternative model for testing antitumour therapies.

Evaluating cell viability in three-dimensional (3D) bioprinted models is a crucial step for determining both the functionality and biocompatibility of the scaffolds employed. In this study, the combined use of the fluorescent dyes Hoechst-33,342 and SYTOX™ Green proved effective for distinguishing viable from non-viable cells

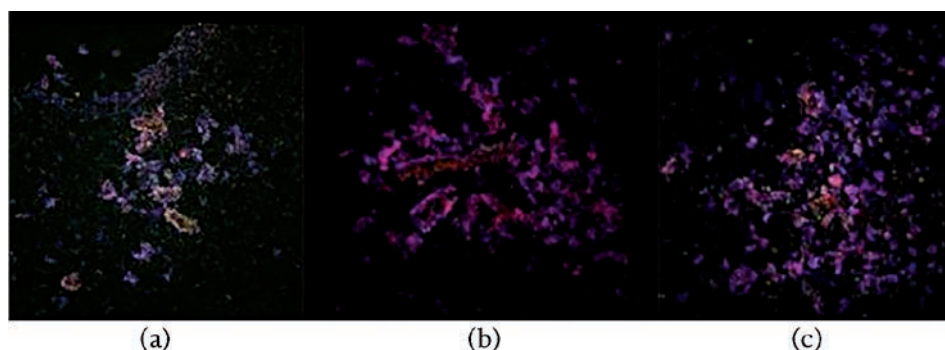


Figure 4.
Viability of tumor spheroids: (A) LNCaP, RWPE-1, and PBMC treated with hPRL; (B) LNCaP and RWPE-1 treated with hPRL without irradiation; (C) LNCaP and RWPE-1 treated with hPRL and exposed to 2 Gy irradiation.

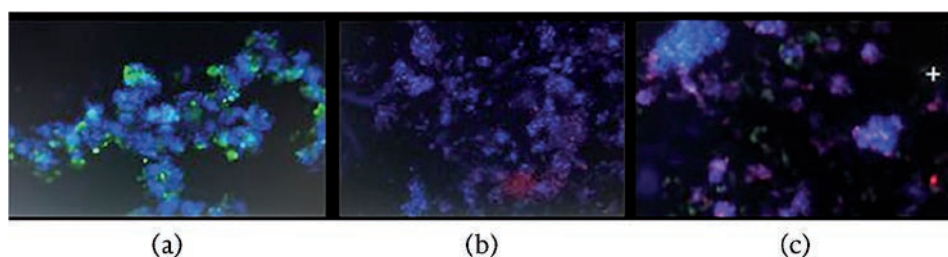


Figure 5.
Viability of LNCaP and RWPE-1 spheroids: (A) treated with hPRL at 24 h; (B, C) untreated with hPRL and exposed to 2 Gy radiation at 48 and 72 h, respectively.

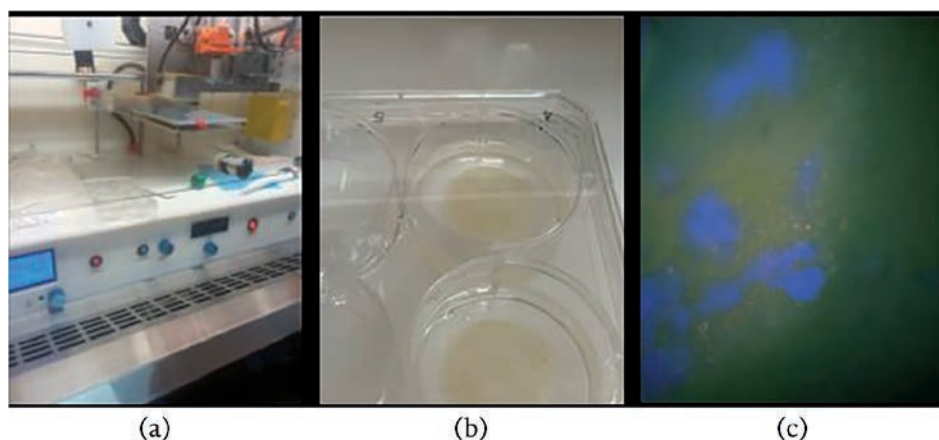


Figure 6.
(A) Bioprinter used for the prostate construct; (B) plate containing constructs in PBS; (C) construct containing LNCaP and RWPE-1 cells stained with Hoechst-33,342 and Sytox Green.

within the hydrogel structure. Upon violet-light excitation, viable cells exhibited the characteristic blue fluorescence (Hoechst), whereas permeabilized, non-viable cells emitted green fluorescence due to SYTOXTM Green incorporation. The additional use of the lipophilic tracers DID and DIO was instrumental for identifying and tracking the spatial distribution and interactions of the two cell populations—LNCaP (tumor) and RWPE-1 (non-tumoural epithelial)—embedded in the hydrogel, highlighting the system's ability to reproduce complex cellular interactions that are typically lost in conventional 2D cultures.

Continuous monitoring over a 14-day period revealed that the sodium alginate-nanocellulose hydrogel effectively supported both LNCaP cell proliferation within the lumen and inner periphery of the construct and RWPE-1 cell viability throughout the matrix. These findings reinforce the suitability of naturally derived biopolymers as 3D scaffolds capable of maintaining an appropriate balance between nutrient diffusion, gas exchange, and mechanical stability—essential factors for preserving tissue-like architecture and long-term cell survival.

From a biotechnological perspective, the ability of this hydrogel to sustain heterogeneous co-cultures indicates promising potential not only in prostate tissue engineering but also as an advanced preclinical platform for drug screening and evaluation of antitumour therapies. The incorporation of both normal (RWPE-1)

and malignant (LNCaP) prostate cell types creates a hybrid microenvironment that better mimics the physiological and pathological interactions found *in vivo*, thereby enabling more accurate analyses of differential responses to biochemical or physical stimuli, including irradiation.

Preliminary results also suggest the need for further irradiation kinetics studies using additional cell lines such as NIH/3 T3 (immortalized murine embryonic fibroblasts), to assess hydrogel integrity and cellular responses under stress conditions. Such experiments would expand the applicability of this bioprinted system to broader biomedical contexts, including tissue repair, wound healing, and radiobiological modeling.

In summary, the ability of the alginate-nanocellulose hydrogel to maintain viable cell populations for up to 14 days demonstrates that the construct possesses both physicochemical and biological properties suitable for use in bioengineering and advanced tumor modeling. These findings establish the platform as a versatile and sustainable tool for translational research, supporting applications ranging from targeted therapy testing and radiobiology to tissue regeneration.

4. Conclusions

Taken together, these findings reinforce that three-dimensional models based on LNCaP cells, combined with bioprinting and prolactin stimulation, represent both a methodological and conceptual milestone. They constitute a watershed that expands the frontiers of prostate cancer research, opening new perspectives for the development of more effective, personalized, and biologically relevant therapies.

Acknowledgements

We would like to express our gratitude to Dr. Mônica Beatriz Mathor for granting access to the Bioprinter from her laboratory at the Radiation Technology Centre (CTER), to Dr. Carlos Roberto Jorge Soares from the Biotechnology Centre (CEBIO) for his text revisions and valuable insights, to the Radiobiology Laboratory (CEBIO) for providing access to the INcell Analyzer for the acquisition and analysis of the images generated by the tumoroids, and to the National Nuclear Energy Commission (CNEN) for making the Gamma Cell available for the irradiation experiments. Finally, we extend our thanks to BioConsulting Laboratórios for the commitment of its researchers in conducting the experiments.

Conflict of interest

The author declare no conflict of interest.

Author details

Patrícia Falcão^{1*} and Carlos Roberto Jorge Soares^{1,2}

1 Federal University of Minas Gerais, UFMG, Belo Horizonte, Brazil

2 Nuclear and Energy Research Institute – IPEN, São Paulo, Brazil

*Address all correspondence to: falcpatricialima@gmail.com

IntechOpen

© 2026 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] INCA - Instituto Nacional do Câncer. 2025
- [2] Ittmann M. Anatomy and histology of the human and murine prostate. *Cold Spring Harbor Perspectives in Medicine*. 2018;**8**(5):1-6
- [3] Shah RB, Zou M. Prostate Biopsy Interpretation: An Illustrated Guide. *Medicine, Medicine (R0)*. Cham: Springer; 2012. pp. 1-10. DOI: 10.1007/978-3-030-13601-7
- [4] Sathianathan NJ, Konety BR, Crook J, Saad F, Lwrentschuk N. Landmarks in prostate cancer. *Nature Reviews Urology*. 2018;**15**(10):627-642. DOI: 10.1038/s41585-018-0060-7
- [5] Wang G, Zhao D, Spring DJ, Depinho RA. Genetics and biology of prostate cancer. *Genes and Development*. 2018;**32**(17-18):1105-1140
- [6] Vakar-Lopez F, True ID. Prostate Cancer: Pathology and Genetics. 3rd ed. Vol. 3. 299-310 p. Elsevier; 2018. DOI: 10.1590/S1413-81232008000100027
- [7] Humphrey PA. Diagnosis of adenocarcinoma in prostate needle biopsy tissue. *Journal of Clinical Pathology*. 2007;**60**(1):35-42
- [8] Humphrey PA. Histopathology of prostate cancer. *Cold Spring Harbor Perspectives in Medicine*. 2017;**7**(10):1-21
- [9] Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA: A Cancer Journal for Clinicians*. 2018;**68**:7-30
- [10] Takeshi N, Ikeda K, Horie-Inoue K, Inoue S. Application of prostate cancer models for preclinical study: Advantages and limitations of cell lines, patient-derived xenografts, and three-dimensional culture of patient-derived cells. *Cells*. 20 Jan 2019;**8**(1):74. DOI: 10.3390/cells8010074
- [11] Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Research*. 2008;**68**:5469-5477
- [12] Chen X, Li Q, Liu X, Liu C, Liu R, Rycak K, et al. Defining a population of stem-like human prostate cancer cells that can generate and propagate castration-resistant prostate cancer. *Clinical Cancer Research*. 2016;**22**:4505-4516
- [13] Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *The New England Journal of Medicine*. 2012;**367**:1187-1197
- [14] Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. *The New England Journal of Medicine*. 2013;**368**:138-148
- [15] de Bono JS, Oudard S, Ozguroglu M, Hansen S, Machiels JP, Kocak I, et al. Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: A randomised open-label trial. *Lancet*. 2010;**376**:1147-1154
- [16] Bluemn EG, Coleman IM, Lucas JM, Coleman RT, Hernandez-Lopez S, Tharakan R, et al. Androgen receptor

pathway-independent prostate cancer is sustained through FGF signaling. *Cancer Cell*. 2017;**32**:474-489

[17] Wang HT, Yao YH, Li BG, Tang Y, Chang JW, Zhang J. Neuroendocrine prostate cancer (NEPC) progressing from conventional prostatic adenocarcinoma: Factors associated with time to development of NEPC and survival from NEPC diagnosis - A systematic review and pooled analysis. *Journal of Clinical Oncology*. 2014;**32**:3383-3390

[18] Horoszewicz JS, Leong SS, Chu TM, Wajsman ZL, Friedman M, Papsidero L, et al. The Incap cell line—A new model for studies on human prostatic carcinoma. *Progress in Clinical and Biological Research*. 1980;**37**:115-132

[19] Mickey DD, Stone KR, Wunderli H, Mickey GH, Vollmer RT, Paulson DF. Heterotransplantation of a human prostatic adenocarcinoma cell line in nude mice. *Cancer Research*. 1977;**37**:4049-4058

[20] Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW. Establishment and characterization of a human prostatic carcinoma cell line (pc-3). *Investigative Urology*. 1979;**17**:16-23

[21] Takayama K, Misawa A, Suzuki T, Takagi K, Hayashizaki Y, Fujimura T, et al. Tet2 repression by androgen hormone regulates global hydroxymethylation status and prostate cancer progression. *Nature Communications*. 2015;**6**:8219

[22] Takayama K, Horie-Inoue K, Suzuki T, Urano T, Ikeda K, Fujimura T, et al. Tacc2 is an androgen-responsive cell cycle regulator promoting androgen-mediated and castration-resistant growth of prostate cancer. *Molecular Endocrinology*. 2012;**26**:748-761

[23] Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nature Medicine*. May 1996;**2**(5):561-566. DOI: 10.1038/nm0596-561

[24] Namekawa T, Ikeda K, Horie-Inoue K, Inoue S. Application of Prostate Cancer Models for Preclinical Study: Advantages and Limitations of Cell Lines, Patient-Derived Xenografts, and Three-Dimensional Culture of Patient-Derived Cells. 2019. DOI: 10.3390/cells8010074

[25] McDermott U, Sharma SV, Dowell L, Greninger P, Montagut C, Lamb J, et al. Identification of genotype-correlated sensitivity to selective kinase inhibitors by using high-throughput tumor cell line profiling. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;**104**:19936-19941

[26] Culig Z, Hoffmann J, Erdel M, Eder IE, Hobisch A, Hittmair A, et al. Switch from antagonist to agonist of the androgen receptor bicalutamide is associated with prostate tumour progression in a new model system. *British Journal of Cancer*. 1999;**81**:242-251

[27] Blumenthal RD, Goldenberg DM. Methods and goals for the use of in vitro and in vivo chemosensitivity testing. *Molecular Biotechnology*. 2007;**35**(2):185-197

[28] Eagle H, Foley GE. Cytotoxicity in human cell cultures as a primary screen for the detection of anti-tumor agents. *Cancer Research*. 1958;**18**(9):1017-1025. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/13596943>

[29] Hirschberg E. Tissue culture in cancer chemotherapy screening. *Cancer*

- Research. 1958;**18**(8 Part 1):869-878. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/13573357>
- [30] Griffith LG, Swartz MA. Capturing complex 3D tissue physiology in vitro. *Nature Reviews Molecular Cell Biology*. 2006;**7**(3):211-224
- [31] Antoni D, Burckel H, Josset E, Noel G. Three-dimensional cell culture: A breakthrough in vivo. *International Journal of Molecular Sciences*. 2015;**16**(12):5517-5527. Available from: <http://www.mdpi.com/1422-0067/16/3/5517>
- [32] Doyle AD, Yamada KM. Mechanosensing via cell-matrix adhesions in 3D microenvironments. *Experimental Cell Research*. 2016;**343**(1):60-66. DOI: 10.1016/j.yexcr.2015.10.033
- [33] Harunaga JS, Yamada KM. Cell-matrix adhesions in 3D. *Matrix Biology*. 2011;**30**(7-8):363-368. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf>
- [34] Lin R-Z, Chang H-Y. Recent advances in three-dimensional multicellular spheroid culture for biomedical research. *Biotechnology Journal*. 2008;**3**(9-10):1285-1285. Available from: <http://doi.wiley.com/10.1002/biot.1285>
- [35] Falcão PL, Soares CRJ, Nunes AP, Menezes F, Dias G, Thais M, et al. IL-6 downregulates mRNA expression of STAT-3 in low-dose irradiated MDAMB-231 cells treated with aqueous extract of propolis (APE). *Revista Radiologia Brasileira*. 2024 (Pre-print)
- [36] Finnberg NK, Gokare P, Lev A, Grivennikov SI, Macfarlane AW, Campbell KS, et al. Application of 3D tumoroid systems to define immune and cytotoxic therapeutic responses based on tumoroid and tissue slice culture molecular signatures. *Oncotarget*. 2017;**8**(40):66747-66757
- [37] Kaushik G, Ponnusamy MP, Batra SK. Concise review: Current status of three-dimensional organoids as preclinical models. *Stem Cells*. 2018;**36**(9):1329-1340
- [38] Bartlett R, Everett W, Lim S, Natasha G, Loizidou M, Jell G, et al. Personalized in vitro cancer modeling — Fantasy or reality? *Translational Oncology*. 2014;**7**(6):657-664. DOI: 10.1016/j.tranon.2014.10.006
- [39] Broutier L, Mastrogiovanni G, Verstegen MMA, Francies HE, Gavarró LM, Bradshaw CR, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. *Nature Medicine*. 2017;**23**(12):1424-1435. DOI: 10.1038/nm.4438
- [40] Fan H, Demirici U, Chen P. Emerging organoid models: Leaping forward in cancer research. *Journal of Hematology and Oncology*. 2019;**12**(1):1-10
- [41] Kimn M, Mun H, Sung CO, Cho EJ, Jeon HJ, Chun SM, et al. Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening. *Nature Communications*. 5 Sep 2019;**10**(1):3991. DOI: 10.1038/s41467-019-11867-6
- [42] Da Silva GD, Falcão PL, Lima MP, Silva TM, Santos EC, Prudente SR, et al. Transformation of cellulose fibers through electron beam irradiation for the production of hydrogels for the preparation of 3D cell cultures. In: 2024 International Nuclear Atlantic Conference – INAC 2024. Escola de Guerra Naval, Rio de Janeiro, RJ, May 6-10, 20. Congress INAC; 2024.

Section 3

Evolving Therapeutic Paradigms in Renal and Urothelial Carcinomas

Neoadjuvant Immune Checkpoint Inhibitors and Antibody-Drug Conjugates in Urothelial Carcinoma: Current Evidence and Future Perspectives

Kevin Lu

Abstract

Urothelial carcinoma (UC), encompassing muscle-invasive bladder cancer (MIBC) and upper tract urothelial carcinoma (UTUC), remains a therapeutic challenge due to its aggressive biology and high metastatic potential. While cisplatin-based neoadjuvant chemotherapy (NAC) has been the cornerstone of perioperative management, nearly 50% of patients are ineligible due to renal dysfunction or comorbidities, underscoring the urgent need for safer, more effective strategies. Immune checkpoint inhibitors (ICIs) and antibody-drug conjugates (ADCs) have emerged as transformative therapies, with early-phase trials demonstrating promising pathological complete response (pCR) rates and enhanced tolerability. This review synthesizes evidence from pivotal trials evaluating neoadjuvant ICIs and/or ADCs in MIBC and UTUC. The PURE-01 trial reported a pCR rate of 37% with pembrolizumab, correlating with PD-L1 expression and tumor mutational burden (TMB). Similarly, the EV-103 trial demonstrated a 45% pCR rate for enfortumab vedotin combined with pembrolizumab, highlighting the synergistic potential of dual-mechanism regimens. ADCs, leveraging antigen-specific cytotoxicity, minimize systemic toxicity while achieving significant tumor downstaging. Molecular biomarkers are critical for patient stratification. Safety profiles remain manageable, though immune-related adverse events (irAEs) and ADC-specific toxicities necessitate vigilant monitoring. Emerging strategies, such as biomarker-driven combinations and organ-preserving protocols, aim to optimize outcomes while reducing surgical morbidity. In conclusion, neoadjuvant ICIs and ADCs represent a paradigm shift in UC management, offering durable responses and personalized therapeutic avenues. Ongoing trials exploring novel combinations and innovative endpoints will further refine perioperative strategies. By integrating these advances, clinicians can improve survival, preserve organ function, and enhance quality of life for patients with high-risk UC.

Keywords: urothelial carcinoma (UC), immune checkpoint inhibitors (ICIs), antibody-drug conjugates (ADCs), neoadjuvant therapy, pathological response

1. Introduction

Bladder cancer is one of the most common malignancies worldwide, with urothelial carcinoma accounting for over 570,000 new cases and 210,000 deaths every year [1–3]. In 2025, it is estimated that 84,870 new cases of bladder cancer will be diagnosed in the United States, with 17,420 deaths expected, which shows its significant clinical impact [4]. Among the various subtypes of urothelial carcinoma (UC), muscle-invasive bladder cancer (MIBC) is a particularly aggressive form that constitutes approximately 25% of all newly diagnosed cases [2, 4]. MIBC is a particularly aggressive form that tends to progress and metastasize, posing significant therapeutic challenges. In addition to bladder cancer, upper tract urothelial carcinoma (UTUC), which affects the renal pelvis and ureters, is also described and is responsible for 5–10% of all UC cases [3, 5]. Although UTUC is less common, it has similarly poor outcomes, especially in the high-grade or muscle-invasive form, which has a high risk of recurrence and metastasis.

The standard treatment for MIBC and high-risk UTUC includes radical surgery—radical cystectomy (RC) for bladder cancer [6–8] and nephroureterectomy (NU) for UTUC—combined with neoadjuvant chemotherapy [9] (NAC). Cisplatin-based chemotherapy regimens have been shown to improve survival by reducing tumor burden and addressing micrometastatic disease before surgery [10]. Despite its benefits, cisplatin-based NAC is contraindicated in nearly half of MIBC/UTUC patients due to renal dysfunction, advanced age, or comorbidities, limiting its applicability. These limitations underscore the urgent need for alternative neoadjuvant strategies that are effective and well-tolerated in cisplatin-ineligible patients (**Figure 1**) [11–13].

Recent advancements in cancer immunotherapy have introduced a new paradigm in the management of UC (**Figure 2**) [14–22]. Immune checkpoint inhibitors (ICIs) targeting key pathways such as programmed death-1 (PD-1), programmed death ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have revolutionized the treatment landscape of multiple malignancies. In metastatic

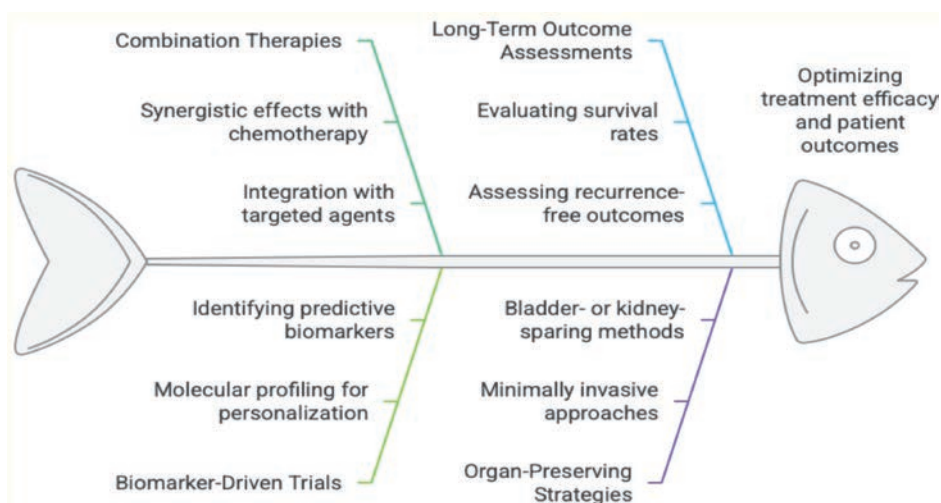


Figure 1.
Enhancing neoadjuvant treatment for urothelial cancer.

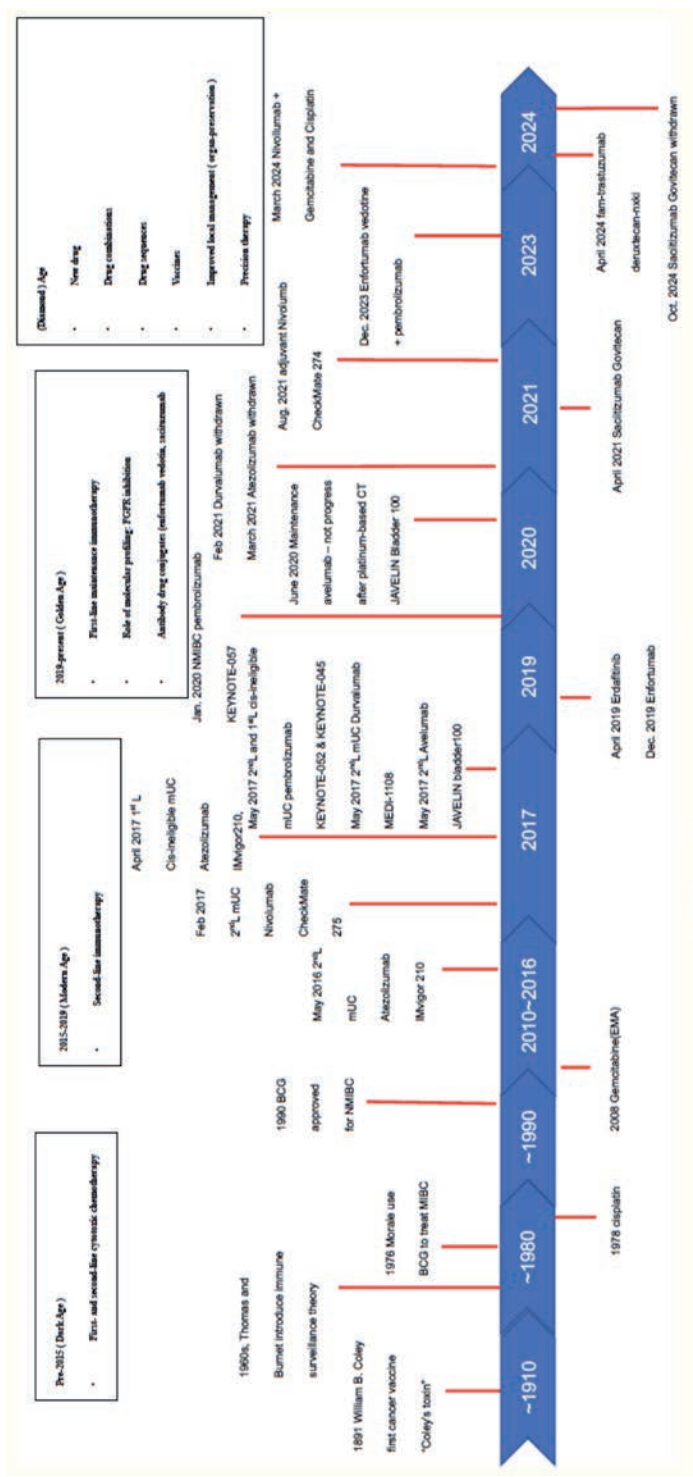


Figure 2.
Treatment landscape of urothelial cancer. ADC: antibody-drug conjugate; BCG: Bacillus Calmette-Guerin; FGFR: fibroblast growth factor receptor; NMIBC: non-muscle invasive bladder cancer; MIBC: muscle-invasive bladder cancer; UC: urothelial carcinoma.

urothelial carcinoma (mUC), ICIs have shown durable responses and significant survival benefits, leading to their approval in both first-line and subsequent treatment settings [23–25]. Inspired by these successes, investigators have turned their attention to exploring the utility of ICIs in earlier stages of UC, including the neoadjuvant setting for both MIBC and UTUC [5, 26–30]. The rationale for this approach lies in the idea that ICIs can induce robust immune responses against tumor cells when the tumor microenvironment is intact, which may result in better pathological responses and long-term outcomes [31].

In parallel with the advancements in immunotherapy, antibody-drug conjugates (ADCs) have emerged as a novel and promising class of targeted therapies in UC [32–34]. ADCs consist of monoclonal antibodies conjugated to potent cytotoxic agents, enabling selective delivery to tumor cells expressing specific antigens. This targeted approach not only enhances the efficacy of the cytotoxic payload but also minimizes off-target effects, thereby improving the therapeutic index. Two ADCs, enfortumab vedotin and sacituzumab govitecan, have demonstrated significant clinical activity in advanced urothelial carcinoma [35]. Enfortumab vedotin targets Nectin-4, a cell adhesion molecule highly expressed in UC [36–41], while sacituzumab govitecan targets Trop-2, a transmembrane glycoprotein overexpressed in many epithelial cancers [42–45]. Given their success in advanced disease, these agents are now being investigated in the neoadjuvant setting for both MIBC and UTUC, either alone or in combination with ICIs, to further improve pathological responses and survival outcomes.

The integration of ICIs and ADCs in the neoadjuvant treatment of UC represents a paradigm shift in the management of this disease [29, 30, 46]. Unlike traditional chemotherapy, which exerts its effects primarily through direct cytotoxicity, ICIs and ADCs offer unique mechanisms of action that may work synergistically to enhance tumor eradication. ICIs primarily activate the immune system to target cancer cells, while ADCs deliver cytotoxic agents directly to tumor cells, minimizing systemic toxicity compared to traditional chemotherapy. This combination has the potential to achieve deeper pathological responses and address both local and micrometastatic disease.

Several key clinical trials have provided insights into the efficacy of neoadjuvant ICIs and ADCs in UC. For example, the PURE-01 trial, which evaluated pembrolizumab in the neoadjuvant setting, reported a pCR rate of 37%, with higher responses observed in patients with PD-L1-positive tumors [47–49]. Similarly, the ABACUS trial, which investigated atezolizumab as a neoadjuvant therapy, demonstrated a pCR rate of 31% [50, 51]. These trials underscore the importance of molecular biomarkers, such as PD-L1 expression and tumor mutational burden (TMB), in predicting responses to ICIs. Meanwhile, early-phase trials evaluating the combination of ADCs with ICIs have shown encouraging results. The EV-103 trial, for instance, explored the use of enfortumab vedotin in combination with pembrolizumab, reporting high response rates and significant tumor shrinkage [37].

The evolving landscape of neoadjuvant therapy in UC presents both opportunities and challenges. On one hand, the integration of ICIs and ADCs offers the potential to improve outcomes for patients who are ineligible for traditional NAC or who may benefit from a more personalized approach to treatment. On the other hand, these novel therapies introduce new considerations regarding patient selection, toxicity management, and cost-effectiveness. Identifying reliable predictive biomarkers is crucial to optimizing patient selection and maximizing the benefits of neoadjuvant

ICIs and ADCs. Furthermore, understanding the safety profiles of these agents in the neoadjuvant setting is essential to ensure that their use does not compromise surgical outcomes or increase perioperative risks.

This review aims to provide a comprehensive and practice-oriented analysis of the current evidence on neoadjuvant ICIs and ADCs in urothelial carcinoma. We highlight key clinical trials, discuss pathological response rates, explore molecular predictors of response, and address safety considerations. Additionally, we examine future directions in the field, including the potential for combination strategies and bladder- or kidney-preserving approaches. By synthesizing the latest research and clinical insights, we aim to equip clinicians with the knowledge needed to develop tailored, patient-specific treatment strategies that optimize oncologic outcomes and improve quality of life for patients with MIBC and UTUC. This review focuses on neoadjuvant ICIs and ADCs in cisplatin-eligible and ineligible patients, emphasizing molecular biomarkers, combination strategies, and emerging organ-preserving approaches.

2. Historical perspective on neoadjuvant therapies for urothelial carcinoma

The concept of neoadjuvant therapy in UC emerged from the need to improve survival outcomes for patients undergoing RC [8, 52–54]. Early studies demonstrated that cisplatin-based NAC achieves pathological downstaging and improves overall survival in some patients [14, 55–58]. The Southwest Oncology Group (SWOG) 8710 trial reported a 5-year survival rate of 57% with the MVAC regimen (methotrexate, vinblastine, doxorubicin, and cisplatin) versus 43% with surgery alone in MIBC, albeit with significant hematologic and gastrointestinal toxicities.

However, the use of NAC is limited by its toxicity profile and the high proportion of patients who are ineligible for cisplatin-based regimens due to renal dysfunction, poor performance status, or other comorbidities [59]. These limitations have driven the exploration of ICIs and ADCs as less toxic neoadjuvant alternatives [60–63].

2.1 Neoadjuvant immune checkpoint inhibition in urothelial carcinoma

The use of ICI in the neoadjuvant setting for UC is driven by their ability to modulate the immune system to recognize and attack tumor cells [64]. By inhibiting key immune checkpoint pathways, ICIs restore and enhance T-cell-mediated immune responses, offering a promising strategy to improve outcomes in MIBC and UTUC [17, 18, 65]. The growing interest in neoadjuvant ICIs is supported by several pivotal clinical trials that have demonstrated encouraging pCR rates and downstaging outcomes in MIBC.

2.1.1 Key clinical trials on neoadjuvant ICIs

2.1.1.1 PURE-01 trial (NCT02736266)

The PURE-01 trial was a landmark phase II study that evaluated pembrolizumab as a neoadjuvant therapy in MIBC. The trial enrolled 50 patients who received three cycles of pembrolizumab before radical cystectomy.

Results: Primary endpoint: pCR rate (37%); secondary endpoints: downstaging rate (55%) [47–49].

2.1.1.2 ABACUS trial (NCT02662309)

The ABACUS trial was a phase II study that assessed atezolizumab as a neoadjuvant therapy in MIBC. The trial enrolled 95 patients who received two cycles of atezolizumab before RC.

Results: Primary endpoint: pCR rate (31%); exploratory analysis: PD-L1 correlation [50, 51].

2.1.1.3 NABUCCO trial (NCT03387761)

The NABUCCO trial explored a dual checkpoint blockade using ipilimumab (anti-CTLA-4) and nivolumab (anti-PD-1) in the neoadjuvant setting for MIBC.

Results: Primary endpoint: pCR rate (46%); safety: Grade 3–4 irAEs in 32% [66–68].

The use of ICIs in the neoadjuvant setting represents a promising advancement in the management of urothelial carcinoma. Clinical trials such as PURE-01, ABACUS, and NABUCCO have demonstrated that neoadjuvant ICIs can achieve significant pathological responses, particularly in patients with high PD-L1 expression or elevated TMB. By targeting immune checkpoints, ICIs activate the immune system to address both the primary tumor and micrometastatic disease, potentially improving surgical outcomes and long-term survival. As ongoing trials continue to refine the role of ICIs in neoadjuvant therapy, their integration into clinical practice holds the potential to transform the treatment landscape for patients with MIBC and UTUC.

2.2 Neoadjuvant antibody-drug conjugates in urothelial carcinoma

ADCs represent a novel and promising class of targeted therapies that combine the specificity of monoclonal antibodies with the potency of cytotoxic agents. Their application in the neoadjuvant setting aims to maximize tumor cell eradication while minimizing systemic toxicity, making them an attractive option for patients with MIBC and UTUC who may not tolerate traditional chemotherapy.

2.2.1 Enfortumab vedotin

Enfortumab vedotin targets Nectin-4, a cell surface protein highly expressed in urothelial carcinoma. It delivers monomethyl auristatin E (MMAE), a potent microtubule-disrupting agent that induces tumor cell apoptosis.

Mechanism of Action: Enfortumab vedotin binds to Nectin-4 on tumor cells, leading to internalization of the ADC and release of MMAE, which disrupts microtubules and triggers cell death.

Clinical Evidence: The EV-301 trial (NCT03474107) demonstrated significant survival benefits in previously treated mUC, establishing enfortumab vedotin as a key therapeutic option [36, 41]. The ongoing KEYNOTE-869/EV-103 phase 1/2 trial (NCT03288545) is now evaluating its use in the neoadjuvant setting, with preliminary results suggesting promising tumor regression and pathological responses [37].

2.2.2 Sacituzumab govitecan

Sacituzumab govitecan targets Trop-2, a transmembrane glycoprotein overexpressed in many epithelial cancers, including urothelial carcinoma. It delivers SN-38, an active metabolite of the topoisomerase I inhibitor irinotecan, to tumor cells [43].

Mechanism of Action: Upon binding to Trop-2, sacituzumab govitecan is internalized by tumor cells, releasing SN-38, which causes DNA damage and cell death.

Clinical Evidence: The TROPHY-U-01 trial (NCT03547973) reported high response rates in advanced UC, particularly in patients who had progressed on prior therapies [42, 44, 45, 69]. Ongoing phase 2 SURE-01 study (NCT05226117) is investigating its potential in the neoadjuvant setting, aiming to enhance pCR rates while reducing systemic toxicity.

2.3 The rationale for neoadjuvant immune checkpoint inhibition and antibody-drug conjugates in urothelial carcinoma

The integration of ICIs and ADCs in neoadjuvant regimens represents a transformative advancement in the management of UC. These therapies, with distinct but complementary mechanisms of action, offer the potential to enhance pathological responses, minimize systemic toxicity, and improve long-term outcomes in patients with MIBC and UTUC.

2.3.1 Immune checkpoint inhibitors

ICIs restore and amplify T-cell-mediated immune responses by blocking inhibitory checkpoint pathways, such as PD-1, PD-L1, and CTLA-4. In the neoadjuvant setting, ICIs harness the intact tumor microenvironment to prime the immune system, enhancing its ability to recognize and eradicate tumor cells. This immune activation reduces tumor burden, addresses micrometastatic disease early, and promotes durable pathological responses, ultimately improving surgical outcomes and long-term survival.

2.3.2 Antibody-drug conjugates

ADCs leverage the specificity of monoclonal antibodies to selectively deliver potent cytotoxic agents to tumor cells expressing specific surface antigens. By targeting cancer cells with precision, ADCs minimize off-target effects and systemic toxicity compared to conventional chemotherapy. In the neoadjuvant context, ADCs significantly reduce tumor burden prior to surgery, enhancing pathological responses and increasing the likelihood of successful surgical resection.

2.3.2.1 Synergistic potential of ICIs and ADCs

The combination of ICIs and ADCs in neoadjuvant regimens addresses both immune evasion and residual tumor burden [28, 46, 70]. ICIs activate the immune system to target cancer cells, while ADCs provide direct cytotoxicity to antigen-expressing cells. Together, these therapies achieve synergistic effects, optimizing local and systemic disease control, reducing recurrence rates, and improving overall patient outcomes. This dual approach has positioned the combination of ICIs and ADCs as a cornerstone of personalized treatment strategies in UC.

2.3.2.2 Key combination trials

- KEYNOTE-B15/EV-304 trial (NCT03288545): A randomized phase 3 study exploring the combination of enfortumab vedotin and pembrolizumab in the perioperative setting for MIBC.
- SURE-02 study (NCT05535218): Evaluating neoadjuvant pembrolizumab and sacituzumab govitecan followed by adjuvant pembrolizumab in MIBC.
- NIAGARA study (NCT03732677): Assessing the combination of durvalumab with gemcitabine and cisplatin in cisplatin-eligible MIBC, demonstrating improved pCR and event-free survival [71].
- VOLGA study (NCT04960709): Investigating durvalumab, tremelimumab, and enfortumab vedotin in cisplatin-ineligible patients, showing promising pCR rates.

2.3.2.3 Clinical implications

By integrating ICIs and ADCs into neoadjuvant regimens, clinicians can adopt a dual-pronged strategy that optimizes tumor control and addresses systemic disease. These therapies offer a novel approach for cisplatin-eligible and ineligible patients, providing new avenues for treatment personalization based on molecular and clinical characteristics. As ongoing trials continue to refine these strategies, the synergistic interaction between ICIs and ADCs represents a pivotal advancement in improving survival and quality of life for patients with high-risk UC.

3. Pathological complete response and downstaging rates

pCR is a critical endpoint in neoadjuvant trials, as it is strongly associated with improved survival outcomes (**Figures 3 and 4**). Achieving a pCR indicates the complete eradication of invasive cancer from the surgical specimen, which translates to a better long-term prognosis [29, 72–75]. The integration of ADCs into neoadjuvant regimens has the potential to enhance pCR and tumor downstaging rates beyond those achieved with ICIs alone. ADCs enhance pCR by delivering cytotoxic payloads directly to tumor cells, inducing immunogenic cell death that synergizes with ICIs' immune activation. Preliminary data from ongoing trials suggest promising results, warranting further investigation to optimize these therapeutic approaches.

The pCR rates observed in neoadjuvant ICI trials are encouraging when compared to historical data from cisplatin-based NAC [76]. Key clinical trials have demonstrated that ICIs, alone or in combination with ADCs, can achieve substantial tumor regression before surgery (substantial tumor regression is defined as downstaging to $\leq$ ypT1N0) (**Table 1**):

The NABUCCO trial highlighted the potential of combination ICI therapy using CTLA-4 and PD-1 inhibitors to achieve higher pCR rates. However, the increased efficacy must be carefully balanced against the risk of immune-related adverse events, which were more frequent with dual checkpoint blockade.

Notably, the EV-103 trial demonstrated that combining enfortumab vedotin, an ADC targeting Nectin-4, with pembrolizumab resulted in a pCR rate of 45% and a

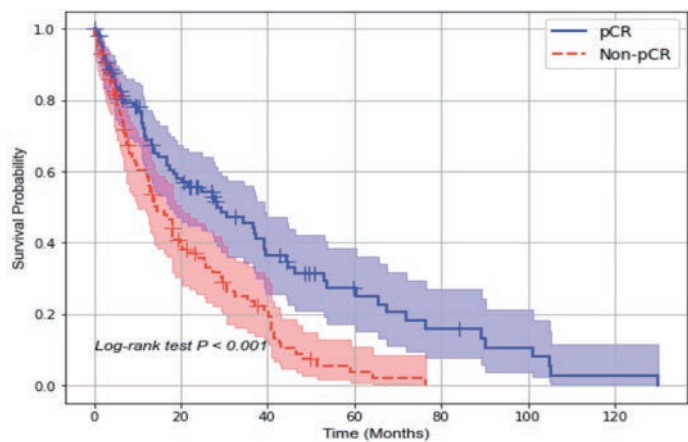


Figure 3.
Kaplan-Meier curves for relapse-free survival (RFS) by pathological complete response (pCR) status: patients who achieved pCR had a hazard ratio for RFS of 0.24 (95% CI, 0.10–0.58, $P < .001$).

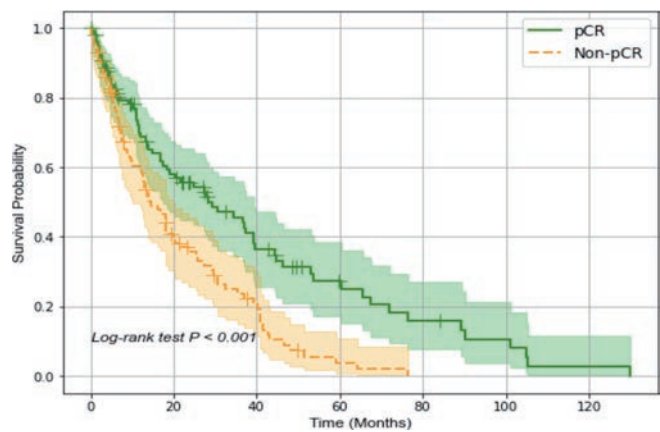


Figure 4.
Kaplan-Meier curves for overall survival (OS) by pathological complete response (pCR) status: patients who achieved pCR had a hazard ratio for OS of 0.19 (95% CI, 0.08–0.40, $P < .001$).

Trial	ICI agent	pCR rate (%)	Downstaging rate (%)	Key biomarkers
PURE-01 [47–49]	Pembrolizumab	37	55	PD-L1, TMB
ABACUS [50, 51]	Atezolizumab	31	48	Immune gene signatures
NABUCCO [66–68]	Ipilimumab + nivolumab	46	58	Immune-related adverse events
EV-103 [37]	Enfortumab vedotin + pembrolizumab	45	60	Nectin-4
SURE-02	Pembrolizumab + sacituzumab govitecan	Pending	Pending	Trop-2

Table 1.
Pathological response rates in key neoadjuvant trials.

downstaging rate of 60%, suggesting that ADCs may synergize with ICIs to enhance tumor regression. The ongoing SURE-02 trial is expected to provide additional insights into the combination of pembrolizumab and sacituzumab govitecan, an ADC targeting Trop-2, which may further improve pCR rates.

These findings underscore the importance of continued exploration of neoadjuvant strategies involving ICIs and ADCs to optimize pathological responses. While higher pCR rates are promising, careful consideration of toxicity profiles and patient selection is essential to maximize therapeutic benefits while minimizing adverse effects.

4. Molecular predictors of response

The identification of predictive biomarkers is crucial for optimizing patient selection and improving the efficacy of neoadjuvant ICIs and/or ADCs in UC. Several key biomarkers have emerged as potential predictors of response, each offering unique insights into tumor biology and treatment outcomes [77–87].

One of the most extensively studied biomarkers is PD-L1 expression, which has been correlated with higher pCR rates in several clinical trials [88, 89]. Tumors with elevated PD-L1 levels may exhibit greater sensitivity to ICIs due to their increased reliance on immune checkpoint pathways for immune evasion. However, the predictive value of PD-L1 remains inconsistent across studies, suggesting that additional factors influence treatment response [90].

TMB is another important biomarker under investigation. Higher TMB reflects the accumulation of mutations that can generate neoantigens, making tumors more immunogenic and susceptible to immune-based therapies [91]. Patients with tumors exhibiting high TMB have demonstrated better responses to ICIs, indicating that this biomarker may help identify candidates most likely to benefit from immunotherapy.

Immune gene signatures, derived from gene expression profiling, offer further insights into the immune landscape of tumors [92]. These signatures can identify immune-active tumors that are more likely to respond to ICIs, particularly those with a robust presence of cytotoxic T-cells and other immune effector cells [93]. Advances in spatial transcriptomics have further enhanced our understanding of the tumor microenvironment by preserving the spatial context of gene expression within tissues. This technology allows for the mapping of immune cell interactions and the identification of localized immune niches, providing critical insights into the spatial distribution of immune-active regions that may predict ICI response [94]. Such profiling may enable more precise patient stratification and personalized treatment approaches.

Molecular subtypes of UC play a significant role in predicting responses to neoadjuvant therapies [95–99]. The basal and luminal subtypes exhibit distinct biological and clinical characteristics that influence their sensitivity to immunotherapy. Basal tumors, characterized by higher levels of immune infiltration and inflammation, have shown greater responsiveness to ICIs. In contrast, luminal tumors may require combination strategies to achieve optimal therapeutic effects [100].

By integrating these molecular predictors into clinical practice, clinicians can tailor neoadjuvant treatment strategies to maximize efficacy and minimize unnecessary toxicity, ultimately improving outcomes for patients with UC.

5. Safety and tolerability of neoadjuvant ICIs and ADCs

While ICIs and ADCs have demonstrated generally manageable safety profiles in clinical trials, their use in the neoadjuvant setting requires careful consideration of potential adverse events. The combination of these therapies may pose unique challenges due to overlapping or additive toxicities.

Immune-related adverse events (irAEs) are commonly associated with ICIs and can affect various organ systems [101]. Common irAEs include fatigue (20–30%), rash (15%), diarrhea (10%), and hypothyroidism (5–10%). Management involves corticosteroids for Grade ≥ 2 events and hormone replacement for endocrinopathies [102–105].

- **Dermatologic toxicities:** Frequently observed irAEs include maculopapular rash, pruritus, and vitiligo. These are typically managed with topical corticosteroids and antihistamines, though severe cases may require systemic immunosuppression.
- **Gastrointestinal toxicities:** Diarrhea and colitis are among the most common adverse events, particularly with CTLA-4 inhibitors. Early intervention with anti-diarrheal agents and corticosteroids is critical in severe cases to prevent complications such as bowel perforation.
- **Endocrine toxicities:** Hypophysitis, thyroiditis (leading to hypothyroidism or hyperthyroidism), and adrenal insufficiency are frequently observed. Lifelong hormone replacement therapy is often necessary for irreversible endocrinopathies.
- **Pulmonary toxicities:** Pneumonitis is a potentially life-threatening irAE requiring early detection via imaging and prompt initiation of corticosteroids. Severe cases may necessitate additional immunosuppressive therapies.
- **Hepatic toxicities:** Immune-mediated hepatitis can lead to significant liver enzyme elevations and hepatocellular damage. Management typically involves corticosteroids and, in refractory cases, mycophenolate mofetil.
- **Neurological toxicities:** Rare but serious events include immune-mediated encephalitis, myasthenia gravis-like syndromes, and Guillain-Barré syndrome, often requiring urgent immunosuppressive therapy.

ADC-related toxicities vary depending on the specific cytotoxic payload used [33]. Enfortumab vedotin, for example, is associated with peripheral neuropathy and skin rash, while sacituzumab govitecan can cause gastrointestinal side effects, including nausea, diarrhea, and neutropenia. These toxicities can impact a patient's quality of life and may necessitate dose adjustments or supportive care measures.

Effective management of these adverse events is critical to optimizing patient outcomes in the neoadjuvant setting. Clinicians must be vigilant in monitoring for early signs of toxicity and proactive in implementing mitigation strategies. The ability to balance efficacy with tolerability will be essential to ensuring that patients derive maximum benefit from neoadjuvant ICIs and ADCs while minimizing treatment-related risks.

6. Future directions

The neoadjuvant treatment landscape for UC is evolving rapidly, driven by ongoing clinical trials exploring innovative strategies to enhance the efficacy of ICIs. Key approaches include combination therapies that pair ICIs with chemotherapy, radiation, or targeted agents to achieve synergistic effects, improving pathological responses and long-term outcomes. Biomarker-driven trials remain essential, incorporating molecular profiling to identify predictive biomarkers such as PD-L1 expression, TMB, and immune gene signatures, which help guide personalized treatment strategies and reduce unnecessary toxicity [106, 107].

A critical focus of current research is assessing long-term outcomes, including recurrence-free and overall survival, to confirm whether early pathological responses translate into durable clinical benefits. Additionally, minimally invasive, organ-preserving strategies are being explored for patients demonstrating significant tumor regression with neoadjuvant ICIs. These bladder- or kidney-sparing approaches offer the potential to improve quality of life by reducing the need for radical surgery without compromising oncologic control.

Looking ahead, personalized neoadjuvant regimens that integrate ICIs with ADCs and other novel agents represent a promising direction. By addressing multiple pathways of tumor resistance, these combination therapies aim to optimize treatment efficacy. Organ-preserving protocols are also gaining momentum as a feasible alternative to radical cystectomy for select patients, potentially reshaping the standard of care.

Embracing these future directions will enable clinicians to develop more effective, personalized, and less invasive treatment strategies for patients with MIBC and UTUC, ultimately improving both survival outcomes and quality of life [108] (Figure 5).

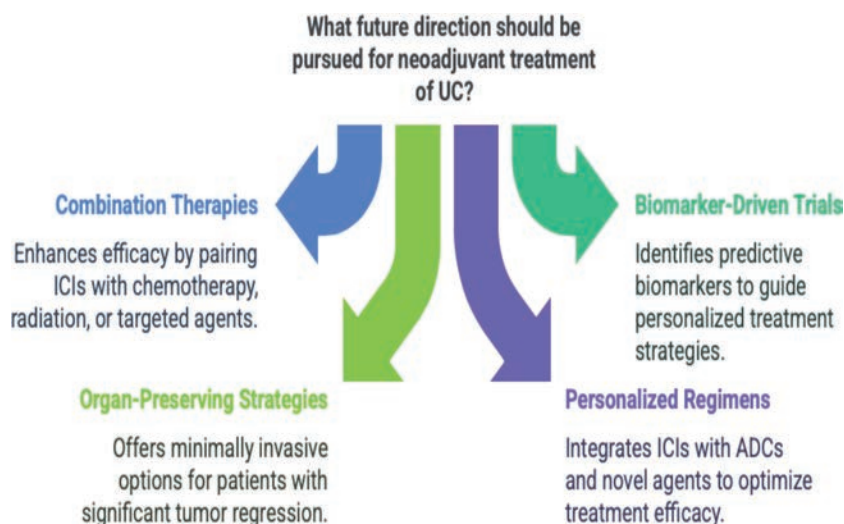


Figure 5.

Future prospect of neoadjuvant immunotherapy for urothelial cancer. ADCs: antibody-drug conjugates; ICIs: immune checkpoint inhibitors; UC: urothelial cancer.

7. Conclusion

The integration of neoadjuvant ICIs and ADCs represents a major advancement in the management of UC. These innovative therapies not only enhance survival outcomes by reducing tumor burden before surgery and targeting micrometastatic disease early but also mitigate chemotherapy-related toxicities such as nephrotoxicity and myelosuppression. This is particularly beneficial for cisplatin-ineligible patients, expanding treatment options while preserving quality of life. The future of UC therapy is evolving toward personalized immunotherapy and targeted approaches, including CAR T-cell therapy, bispecific antibodies, tumor-infiltrating lymphocyte therapies, and biomarker-driven combinations such as FGFR3 inhibitors in luminal tumors. By leveraging these advancements, clinicians can work toward more effective, less invasive, and patient-centered treatment strategies for MIBC and UTUC.

Despite the transformative potential of ICIs and ADCs in the neoadjuvant setting, several challenges persist, including the lack of validated predictive biomarkers (e.g., PD-L1 heterogeneity), financial constraints limiting genomic profiling, and the dynamic evolution of tumor biology during treatment. Overcoming these hurdles requires the development of multi-omic panels and cost-effective, scalable assays to refine patient selection and optimize treatment efficacy. While concerns regarding toxicity and biomarker-driven therapy remain, the synergistic combination of ICIs and ADCs offers a promising pathway to improved outcomes in high-risk UC. Ongoing clinical trials are crucial in further refining these strategies, defining their role in enhancing pathological response rates, and ultimately improving long-term survival for patients with UC.

Author details

Kevin Lu^{1,2}

1 College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

2 Department of Urology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

*Address all correspondence to: kevinlu0620@mail2000.com.tw

IntechOpen

© 2026 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] Filho AM, Laversanne M, Ferlay J, et al. The GLOBOCAN 2022 cancer estimates: Data sources, methods, and a snapshot of the cancer burden worldwide. *International Journal of Cancer*. 2025;**156**(7):1336-1346
- [2] Bray F 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
- [3] Teoh JY et al. Global trends of bladder cancer incidence and mortality, and their associations with tobacco use and gross domestic product per capita. *European Urology*. 2020;**78**(6):893-906
- [4] Siegel RL, Kratzer TB, Giaquinto AN, et al. Cancer statistics, 2025. *CA: A Cancer Journal for Clinicians*. 2025;**75**(1):10-45
- [5] Roumigué M et al. French AFU Cancer Committee Guidelines—Update 2024-2026: Muscle-invasive bladder cancer (MIBC). *The French Journal of Urology*. 2024;**34**(12):102741
- [6] Yin M et al. Neoadjuvant chemotherapy for muscle-invasive bladder cancer: A systematic review and two-step meta-analysis. *The Oncologist*. 2016;**21**(6):708-715
- [7] Traboulsi SL, Kassouf W. A review of neoadjuvant and adjuvant chemotherapy for nonmetastatic muscle invasive bladder cancer. *Urology Practice*. 2016;**3**(1):41-49
- [8] Grossman HB et al. Neoadjuvant chemotherapy plus cystectomy compared with cystectomy alone for locally advanced bladder cancer. *The New England Journal of Medicine*. 2003;**349**(9):859-866
- [9] Oswald D, Pallauf M, Deininger S, et al. Neoadjuvant chemotherapy before nephroureterectomy in high-risk upper tract urothelial cancer: A systematic review and meta-analysis. *Cancers (Basel)*. 2022;**14**(19):4841
- [10] Pfister C et al. Dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin or gemcitabine and cisplatin as perioperative chemotherapy for patients with nonmetastatic muscle-invasive bladder cancer: Results of the GETUG-AFU V05 VESPER trial. *Journal of Clinical Oncology*. 2022;**40**(18):2013-2022
- [11] Roviello G et al. Neoadjuvant treatment in muscle-invasive bladder cancer: From the beginning to the latest developments. *Frontiers in Oncology*. 2022;**12**:912699
- [12] Ruiz de Porras V et al. Neoadjuvant therapy for muscle-invasive bladder cancer: Current clinical scenario, future perspectives, and unsolved questions. *Critical Reviews in Oncology/Hematology*. 2022;**178**:103795
- [13] Font A et al. The challenge of managing bladder cancer and upper tract urothelial carcinoma: A review with treatment recommendations from the Spanish Oncology Genitourinary Group (SOGUG). *Targeted Oncology*. 2019;**14**(1):15-32
- [14] Knollman H et al. Muscle-invasive urothelial bladder cancer: An update on systemic therapy. *Therapeutic Advances in Urology*. 2015;**7**(6):312-330
- [15] Aragon-Ching JB, Trump DL. Systemic therapy in muscle-invasive and

- p>metastatic bladder cancer: Current trends and future promises.
- Future Oncology*
- . 2016;
- 12**
- (17):2049-2058
- [16] Morales-Barrera R et al. Targeting fibroblast growth factor receptors and immune checkpoint inhibitors for the treatment of advanced bladder cancer: New direction and New Hope. *Cancer Treatment Reviews*. 2016;**50**:208-216
- [17] Zibelman M, Plimack ER. Systemic therapy for bladder cancer finally comes into a new age. *Future Oncology*. 2016;**12**(19):2227-2242
- [18] González Del Alba A et al. Recent advances in genitourinary tumors: A review focused on biology and systemic treatment. *Critical Reviews in Oncology/Hematology*. 2017;**113**:171-190
- [19] Gupta S, Gill D, Poole A, Agarwal N. Systemic immunotherapy for urothelial cancer: Current trends and future directions. *Cancers (Basel)*. 2017;**9**(2):15
- [20] Pinto IG. Systemic therapy in bladder cancer. *Indian Journal of Urology*. 2017;**33**(2):118-126
- [21] Eulitt PJ, Bjurlin MA, Milowsky MI. Perioperative systemic therapy for bladder cancer. *Current Opinion in Urology*. 2019;**29**(3):220-226
- [22] Zhang JH, Starr SL, Chamie K. Contemporary systemic therapies in urothelial carcinoma. *Urology*. 2023;**174**:150-158
- [23] Roviello G et al. The evolving treatment landscape of metastatic urothelial cancer. *Nature Reviews. Urology*. 2024;**21**(10):580-592
- [24] Moussa MJ, Campbell MT, Alhalabi O. Revisiting treatment of metastatic urothelial cancer: Where do cisplatin and platinum ineligibility criteria stand? *Biomedicine*. 2024;**12**(3):519
- [25] Einerhand SMH, van der Heijden MS. Advancements in the front-line treatment of metastatic urothelial carcinoma. *European Urology Focus*. 2024;**10**(6):972-974
- [26] Mittal K, Joshi M. Perioperative immunotherapy in urothelial carcinoma: AMBASSADOR charts the path forward. *Med*. 2024;**5**(12):1449-1451
- [27] Robert G, Ravaud A, Gross-Goupil M. How immunotherapy has redefined the treatment paradigm of metastatic or locally advanced muscle-invasive urothelial bladder carcinoma. *Cancers (Basel)*. 2024;**16**(9):1780
- [28] Singh A, Osbourne AS, Koshkin VS. Perioperative immunotherapy in muscle-invasive bladder cancer. *Current Treatment Options in Oncology*. 2023;**24**(9):1213-1230
- [29] Kolawa A, D'Souza A, Tulpule V. Overview, diagnosis, and perioperative systemic therapy of upper tract urothelial carcinoma. *Cancers (Basel)*. 2023;**15**(19):4813
- [30] Kim KH et al. Perioperative systemic therapy in muscle invasive bladder cancer: Current standard method, biomarkers and emerging strategies. *Investigative and Clinical Urology*. 2023;**64**(3):202-218
- [31] Boutaleb I, Mjaess G, Roumeguère T. Perioperative chemotherapy and immunotherapy to optimize cystectomy outcomes in the curative intent of non-metastatic muscle-invasive bladder cancer: A systematic review and meta-analysis. *The French Journal of Urology*. 2024;**34**(9):102704

- [32] Sigorski D et al. Antibody-drug conjugates in uro-oncology. *Targeted Oncology*. 2022;**17**(3):203-221
- [33] Padua TC et al. Efficacy and toxicity of antibody-drug conjugates in the treatment of metastatic urothelial cancer: A scoping review. *Urologic Oncology*. 2022;**40**(10):413-423
- [34] Nathan P et al. Antibody-drug conjugates in the treatment of genitourinary cancers: An updated review of data. *Current Oncology*. 2024;**31**(4):2316-2327
- [35] Bantounou MA et al. Enfortumab vedotin and pembrolizumab as monotherapies and combination treatment in locally advanced or metastatic urothelial carcinoma: A narrative review. *Current Urology*. 2023;**17**(4):271-279
- [36] Powles T et al. Enfortumab vedotin in previously treated advanced urothelial carcinoma. *The New England Journal of Medicine*. 2021;**384**(12):1125-1135
- [37] O'Donnell PH et al. Enfortumab vedotin with or without pembrolizumab in cisplatin-ineligible patients with previously untreated locally advanced or metastatic urothelial cancer. *Journal of Clinical Oncology*. 2023;**41**(25):4107-4117
- [38] Bellmunt J, Nadal R. Enfortumab vedotin and pembrolizumab combination as a relevant game changer in urothelial carcinoma: What is left behind? *Med*. 2024;**5**(6):490-492
- [39] Benjamin DJ, Rezazadeh Kalebasty A, Prasad V. The overall survival benefit in EV-302: Is enfortumab vedotin plus pembrolizumab the new frontline standard of care for metastatic urothelial carcinoma? *European Urology Oncology*. 2024;**7**(3):313-315
- [40] Powles T et al. Enfortumab vedotin and pembrolizumab in untreated advanced urothelial cancer. *The New England Journal of Medicine*. 2024;**390**(10):875-888
- [41] Rosenberg JE et al. EV-301 long-term outcomes: 24-month findings from the phase III trial of enfortumab vedotin versus chemotherapy in patients with previously treated advanced urothelial carcinoma. *Annals of Oncology*. 2023;**34**(11):1047-1054
- [42] Loriot Y et al. TROPHY-U-01, a phase II open-label study of sacituzumab govitecan in patients with metastatic urothelial carcinoma progressing after platinum-based chemotherapy and checkpoint inhibitors: Updated safety and efficacy outcomes. *Annals of Oncology*. 2024;**35**(4):392-401
- [43] Loriot Y et al. Sacituzumab govitecan demonstrates efficacy across tumor Trop-2 expression levels in patients with advanced urothelial cancer. *Clinical Cancer Research*. 2024;**30**(15):3179-3188
- [44] Petrylak DP et al. TROPHY-U-01 cohort 2: A phase II study of sacituzumab govitecan in cisplatin-ineligible patients with metastatic urothelial cancer progressing after previous checkpoint inhibitor therapy. *Journal of Clinical Oncology*. 2024;**42**(29):3410-3420
- [45] Grivas P et al. Sacituzumab govitecan in combination with pembrolizumab for patients with metastatic urothelial cancer that progressed after platinum-based chemotherapy: TROPHY-U-01 cohort 3. *Journal of Clinical Oncology*. 2024;**42**(12):1415-1425
- [46] Mellema JJ, van Rhijn BWG, van der Heijden MS. Recent developments in perioperative combination therapy in muscle-invasive bladder cancer. *Current Opinion in Urology*. 2023;**33**(5):404-411

- [47] Bandini M et al. Predicting the pathologic complete response after neoadjuvant pembrolizumab in muscle-invasive bladder cancer. *Journal of the National Cancer Institute*. 2021;**113**(1):48-53
- [48] Necchi A et al. Updated results of PURE-01 with preliminary activity of neoadjuvant pembrolizumab in patients with muscle-invasive bladder carcinoma with variant histologies. *European Urology*. 2020;**77**(4):439-446
- [49] Necchi A et al. Pembrolizumab as neoadjuvant therapy before radical cystectomy in patients with muscle-invasive urothelial bladder carcinoma (PURE-01): An open-label, single-arm, phase II study. *Journal of Clinical Oncology*. 2018;**36**(34):3353-3360
- [50] Powles T et al. Clinical efficacy and biomarker analysis of neoadjuvant atezolizumab in operable urothelial carcinoma in the ABACUS trial. *Nature Medicine*. 2019;**25**(11):1706-1714
- [51] Szabados B et al. Final results of neoadjuvant atezolizumab in cisplatin-ineligible patients with muscle-invasive urothelial cancer of the bladder. *European Urology*. 2022;**82**(2):212-222
- [52] von der Maase H et al. Gemcitabine and cisplatin versus methotrexate, vinblastine, doxorubicin, and cisplatin in advanced or metastatic bladder cancer: Results of a large, randomized, multinational, multicenter, phase III study. *Journal of Clinical Oncology*. 2000;**18**(17):3068-3077
- [53] Choueiri TK et al. Neoadjuvant dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin with pegfilgrastim support in muscle-invasive urothelial cancer: Pathologic, radiologic, and biomarker correlates. *Journal of Clinical Oncology*. 2014;**32**(18):1889-1894
- [54] Balar AV, Milowsky MI. Neoadjuvant therapy in muscle-invasive bladder cancer: A model for rational accelerated drug development. *The Urologic Clinics of North America*. 2015;**42**(2):217-224, viii-ix
- [55] Jayaratna IS, Navai N, Dinney CP. Risk based neoadjuvant chemotherapy in muscle invasive bladder cancer. *Translational Andrology and Urology*. 2015;**4**(3):273-282
- [56] Leow JJ et al. Perioperative therapy for muscle invasive bladder cancer. *Hematology/Oncology Clinics of North America*. 2015;**29**(2):301-318, ix
- [57] Culine S et al. Chemotherapy for muscle-invasive bladder cancer: Impact of cisplatin delivery on renal function and local control rate in the randomized phase III VESPER (GETUG-AFU V05) trial. *Clinical Genitourinary Cancer*. 2021;**19**(6):554-562
- [58] Schardt J, Roth B, Seiler R. Forty years of cisplatin-based chemotherapy in muscle-invasive bladder cancer: Are we understanding how, who and when? *World Journal of Urology*. 2019;**37**(9):1759-1765
- [59] Jiang DM et al. Defining cisplatin eligibility in patients with muscle-invasive bladder cancer. *Nature Reviews. Urology*. 2021;**18**(2):104-114
- [60] Kluth LA et al. Prognostic and prediction tools in bladder cancer: A comprehensive review of the literature. *European Urology*. 2015;**68**(2):238-253
- [61] Narayan V, Vaughn D. Pharmacokinetic and toxicity considerations in the use of neoadjuvant chemotherapy for bladder cancer. *Expert Opinion on Drug Metabolism & Toxicology*. 2015;**11**(5):731-742
- [62] Sfakianos JP, Galsky MD. Neoadjuvant chemotherapy in the

management of muscle-invasive bladder cancer: Bridging the gap between evidence and practice. *The Urologic Clinics of North America*. 2015;**42**(2):181-187, viii

[63] Einstein DJ, Sonpavde G. Treatment approaches for cisplatin-ineligible patients with invasive bladder cancer. *Current Treatment Options in Oncology*. 2019;**20**(2):12

[64] Raggi D, Moschini M, Necchi A. Neoadjuvant immunotherapy: The next gold standard before radical surgery for urothelial cancer. *European Urology Open Science*. 2021;**30**:34-36

[65] Boegemann M et al. Prospects and progress of immunotherapy for bladder cancer. *Expert Opinion on Biological Therapy*. 2017;**17**(11):1417-1431

[66] van Dorp J et al. High- or low-dose preoperative ipilimumab plus nivolumab in stage III urothelial cancer: The phase 1B NABUCCO trial. *Nature Medicine*. 2023;**29**(3):588-592

[67] Einerhand SMH et al. Survival after neoadjuvant/induction combination immunotherapy vs combination platinum-based chemotherapy for locally advanced (stage III) urothelial cancer. *International Journal of Cancer*. 2022;**151**(11):2004-2011

[68] van Dijk N et al. Preoperative ipilimumab plus nivolumab in locoregionally advanced urothelial cancer: The NABUCCO trial. *Nature Medicine*. 2020;**26**(12):1839-1844

[69] Tagawa ST et al. TROPHY-U-01: A phase II open-label study of sacituzumab govitecan in patients with metastatic urothelial carcinoma progressing after platinum-based chemotherapy and checkpoint inhibitors. *Journal of Clinical Oncology*. 2021;**39**(22):2474-2485

[70] Kim IH, Lee HJ. Perioperative systemic treatment for muscle-invasive bladder cancer: Current evidence and future perspectives. *International Journal of Molecular Sciences*. 2021;**22**(13):7201

[71] Powles T et al. Perioperative durvalumab with neoadjuvant chemotherapy in operable bladder cancer. *The New England Journal of Medicine*. 2024;**391**(19):1773-1786

[72] Pignot G et al. ypT0N0 after neoadjuvant chemotherapy and cystectomy for muscle-invasive bladder cancer: Incidence and prognosis. A review from the Bladder group of the French Committee of Oncology. *Progrès en Urologie*. 2018;**28**(12):567-574

[73] Eule CJ et al. Neoadjuvant cisplatin-based chemotherapy in nonmetastatic muscle-invasive bladder cancer: A systematic review and pooled meta-analysis to determine the preferred regimen. *Urology*. 2024;**188**:118-124

[74] Kim HS et al. Pathological T0 following cisplatin-based neoadjuvant chemotherapy for muscle-invasive bladder cancer: A network meta-analysis. *Clinical Cancer Research*. 2016;**22**(5):1086-1094

[75] Schober JP et al. The past, present, and future of pT0 in bladder cancer clinical trials. *Current Opinion in Urology*. 2022;**32**(5):495-499

[76] Chang E et al. Refining neoadjuvant therapy clinical trial design for muscle-invasive bladder cancer before cystectomy: A joint US Food and Drug Administration and Bladder Cancer Advocacy Network workshop. *Nature Reviews. Urology*. 2022;**19**(1):37-46

[77] Motterle G et al. Predicting response to neoadjuvant chemotherapy in bladder

cancer. *European Urology Focus*. 2020;**6**(4):642-649

[78] Tripathi N et al. Predictors of response to neoadjuvant therapy in urothelial cancer. *Critical Reviews in Oncology/Hematology*. 2024;**194**:104236

[79] Wezel F, Vallo S, Roghmann F. Do we have biomarkers to predict response to neoadjuvant and adjuvant chemotherapy and immunotherapy in bladder cancer? *Translational Andrology and Urology*. 2017;**6**(6):1067-1080

[80] Buttigliero C et al. Molecular biomarkers to predict response to neoadjuvant chemotherapy for bladder cancer. *Cancer Treatment Reviews*. 2017;**54**:1-9

[81] McConkey DJ, Choi W, Dinney CP. Genetic subtypes of invasive bladder cancer. *Current Opinion in Urology*. 2015;**25**(5):449-458

[82] Klaile Y et al. Variant histology in bladder cancer: How it should change the management in non-muscle invasive and muscle invasive disease? *Translational Andrology and Urology*. 2016;**5**(5):692-701

[83] Dimitrov G, Mangaldzhiev R, Slavov C, Popov E. Contemporary molecular markers for predicting systemic treatment response in urothelial bladder cancer: A narrative review. *Cancers (Basel)*. 2024;**16**(17):3056

[84] Crupi E et al. Circulating tumor DNA as a predictive and prognostic biomarker in the perioperative treatment of muscle-invasive bladder cancer: A systematic review. *European Urology Oncology*. 2024;**7**(1):44-52

[85] Bedore S, van der Eerden J, Boghani F, Lokeshwar VB, et al. Protein-based predictive biomarkers

to personalize neoadjuvant therapy for bladder cancer—A systematic review of the current status. *International Journal of Molecular Sciences*. 2024;**25**(18):9899

[86] Lonati C et al. Micropapillary bladder cancer: An evolving biology. *Current Opinion in Urology*. 2022;**32**(5):504-510

[87] Hammouz RY, Kołat D, Kałuzińska Ż, et al. MicroRNAs: Their role in metastasis, angiogenesis, and the potential for biomarker utility in bladder carcinomas. *Cancers (Basel)*. 2021;**13**(4):891

[88] Germanà E, Pepe L, Pizzimenti C, et al. Programmed cell death ligand 1 (PD-L1) Immunohistochemical expression in advanced urothelial bladder carcinoma: An updated review with clinical and pathological implications. *International Journal of Molecular Sciences*. 2024;**25**(12):6750

[89] Rouanne M et al. PD-L1 testing in urothelial bladder cancer: Essentials of clinical practice. *World Journal of Urology*. 2021;**39**(5):1345-1355

[90] Rizzo A, Mollica V, Massari F. Expression of programmed cell death ligand 1 as a predictive biomarker in metastatic urothelial carcinoma patients treated with first-line immune checkpoint inhibitors versus chemotherapy: A systematic review and meta-analysis. *European Urology Focus*. 2022;**8**(1):152-159

[91] Eturi A, Bhasin A, Zarrabi KK, Tester WJ. Predictive and prognostic biomarkers and tumor antigens for targeted therapy in urothelial carcinoma. *Molecules*. 2024;**29**(8):1896

[92] Beckabir W et al. Immune features are associated with response to neoadjuvant chemo-immunotherapy for

muscle-invasive bladder cancer. *Nature Communications*. 2024;**15**(1):4448

[93] Reike MJ et al. Distinct gene expression patterns identify patients who relapse after neoadjuvant pembrolizumab and radical cystectomy in the PURE-01 study. *Clinical Genitourinary Cancer*. 2024;**22**(6):102214

[94] van Dijk N et al. The tumor immune landscape and architecture of tertiary lymphoid structures in urothelial cancer. *Frontiers in Immunology*. 2021;**12**:793964

[95] Kujtan L et al. The evolving genomic landscape in urothelial cancer. *Current Opinion in Oncology*. 2018;**30**(3):197-202

[96] Matulay JT, Kamat AM. Advances in risk stratification of bladder cancer to guide personalized medicine. *F1000Res*. 2018;**7**:F1000 Faculty Rev-1137

[97] Lerner SP et al. Association of molecular subtypes with pathologic response, PFS, and OS in a phase II study of COXEN with neoadjuvant chemotherapy for muscle-invasive bladder cancer. *Clinical Cancer Research*. 2024;**30**(2):444-449

[98] Ulapec M et al. New insights into the diagnosis, molecular taxonomy, and treatment of bladder cancer. *Acta Medica Academica*. 2021;**50**(1):143-156

[99] Kardoust Parizi M et al. The value and limitations of urothelial bladder carcinoma molecular classifications to predict oncological outcomes and cancer treatment response: A systematic review and meta-analysis. *Urologic Oncology*. 2021;**39**(1):15-33

[100] Groeneveld CS et al. Basal/squamous and mixed subtype bladder cancers present poor outcomes after neoadjuvant chemotherapy in the

VESPER trial. *Annals of Oncology*. 2025;**36**(1):89-98

[101] Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *The New England Journal of Medicine*. 2018;**378**(2):158-168

[102] Schneider BJ et al. Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: ASCO guideline update. *Journal of Clinical Oncology*. 2021;**39**(36):4073-4126

[103] Brahmer JR, Abu-Sbeih H, Ascierto PA, Brufsky J, Cappelli LC, Cortazar FB, et al. Society for Immunotherapy of Cancer (SITC) clinical practice guideline on immune checkpoint inhibitor-related adverse events. *Journal for Immunotherapy of Cancer*. 2021;**9**(6):e002435

[104] Majem M et al. SEOM clinical guideline for the management of immune-related adverse events in patients treated with immune checkpoint inhibitors (2019). *Clinical & Translational Oncology*. 2020;**22**(2):213-222

[105] Thompson JA et al. NCCN Guidelines® Insights: Management of immunotherapy-related toxicities, version 2.2024. *Journal of the National Comprehensive Cancer Network*. 2024;**22**(9):582-592

[106] Scholtes MP, Alberts AR, Iflé IG, et al. Biomarker-oriented therapy in bladder and renal cancer. *International Journal of Molecular Sciences*. 2021;**22**(6):2832

[107] Nassif EF et al. Precision immunity: Immunoscore and neoadjuvant treatment in bladder cancer. *Oncoimmunology*. 2021;**10**(1):1888488

[108] Pardo JC, Ruiz de Porras V, Plaja A, et al. Moving towards personalized medicine in muscle-invasive bladder cancer: Where are we now and where are we going? *International Journal of Molecular Sciences*. 2020;**21**(17):6271

Perspective Chapter: Current Role of Adjuvant Therapy in High Risk for Recurrence Resected Kidney Cancer

*Lujin Alajmani, Badr Rafi, Hassan Fadil
and Rowaa A. Banjar*

Abstract

Kidney cancer accounts for 3% of global cancer diagnosis. Regardless of cancer stage or histological type, surgical resection is a component of RCC therapy. Unfortunately, few cases of localized renal cell cancer experience post-surgical resection with a 30 to 40% 5-year recurrence risk. Therefore, for long-term disease free survival surgery alone is not enough which illicit the need for further management. In order to reduce the chance of recurrence, adjuvant therapy is used to dress the unidentified residual micro metastatic disease components. Proper risk stratification nomograms need to be used in order to identify patient who will need and benefit from such modality. Many papers emerged studying different treatment modalities targeting various pathways involved in RCC as adjuvant therapy. This chapter will mention all different adjuvant modalities been used including targeted therapy and its emerging data. Clinician should discuss these options with their patients and inform them regarding ongoing trials to reach a joined decision.

Keywords: adjuvant therapy, RCC, localized RCC, targeted therapy, mTOR, TKI, ICI

1. Introduction

Kidney cancer constitutes 3% of all global cancer diagnosis and accounts for 1.8% of global cancer mortality with approximately 431,288 affected individuals per year [1]. Approximately, renal cell carcinoma (RCC) accounts for 90% of all kidney cancers affecting 388,159 individuals annually [2]. 50.2 of RCC patients are either present or past smokers [2, 3]. A higher risk of RCC is associated with having a first-degree relative with renal cancer [4].

There are three main types of RCC that have been confirmed by genetic and cytogenic analysis: papillary RCC (pRCC), clear cell RCC (ccRCC), and chromophobe RCC (chRCC) [2].

Surgical resection of the cancer is part of RCC management regardless of the stage or its histological subtype. Radical or partial nephrectomy is one surgical option [5].

The ideal standard therapy for localized RCC is surgical resection which can be considered curative in cases, unlike metastatic disease.

Unfortunately, few localized RCC cases face post-surgical cancer recurrence with a 5-year recurrence rate of 30 to 40% [6]. Hence, for long-term disease-free survival, surgery alone is not sufficient [6]. The standard of care post nephrectomy for localized RCC currently is history and physical examination, laboratory testing and radiographic surveillance with no rule of adjuvant therapy [7]. Adjuvant therapy is much needed in almost 50% of patients post nephrectomy as they face recurrence with no signs and symptoms [5, 7].

The role of adjuvant therapy is to treat the unrecognized residual micro-metastatic disease bits to decrease the risk of recurrence. Adjuvant therapy use has recently gained popularity in genitourinary tumor and is up to our time is still being explored and studied unlike other tumors [5]. In light of such findings, a strong desire for adjuvant therapy in high recurrence risk patient is based on logic grounds.

Over the past years, a revolution in the management of RCC occurred with the advances of targeted therapy use in RCC. In the early 2000, targeted therapy explored as a result of the discovery of vascular endothelial growth factor pathway and its role in RCC. Agents such as Sorafenib Pazopanib and Sunitinib introduced and used in many trials.

As adjuvant therapy is becoming a popular option to offer patients, in this chapter we review new the evidence of variable post-surgical resection management modalities for non-metastatic that been used.

2. Risk stratification

Identifying patients at high risk of disease recurrence postsurgical resection is a crucial step to determine whether a patient would benefit from the therapy and to effectively test and utilize adjuvant therapy. The most significant prognostic factor in RCC is stage, with 5 years cancer specific survival rates being 91–100%, 74–96%, 59–70%, and 16–32% in stage I, II, III, and IV, respectively. There are multiple nomograms to stratify and predict RCC recurrence rate. Including: Memorial Sloan Kettering Cancer Center (MSKCC) Nomogram, International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) Nomogram, UCLA Integrated Staging System (UISS), Stage, Size, Grade, and Necrosis (SSGN) Nomogram, and Cleveland Clinic Nomogram. These systems are usually composed of various factors and prognostic variables such as pathologic and clinical features (tumor size, histopathologic type, nuclear grade lymphovascular invasion, stage at diagnosis, and surgical margin), molecular and biologic markers, and post-operative factors [5, 7].

The validated and most common used nomograms are UISS and SSGN.

3. Radiation therapy

Patients with metastatic RCC with symptoms like painful bone metastasis and hematuria have been treated with palliative radiotherapy. Moreover, use of radiotherapy in a subgroup of patients who have solitary bone metastasis was reported to positively affect long-term progression free survival [5]. A randomized prospective trial compared the administration of radiotherapy for stage II and III RCC to the kidney bed and lymph nodes; ipsilateral and contralateral lymph nodes versus observation

demonstrated relapse rate of 48% in both groups. Significant complications observed in 44% of patients who received radiotherapy with a mortality rate of 19% [8].

4. Hormonal therapy

RCC is more common in male with a 2:1 male to female ratio suggesting that sex hormones have an influence in the pathogenesis of RCC. Androgens, for instance testosterone and androgen receptors can trigger some signaling pathways and amend the expression of HIF and VEGF. On the other hand, estrogen shown to increase apoptosis and reduces growth of tumor cells [9]. Moreover, medroxy progesterone acetate (MPA) used as hormonal adjuvant with the rationale that some patients with metastatic RCC had positive response to such treatment [5]. One randomized prospective trial on 136 patients compares the use of adjuvant MPA 500 g (three times a week) for a year post-surgical resection versus observation. There were no significant differences in relapses between the observation group and adjuvant group (33.9% vs. 32.7, respectively) with a median of 5 years follow-up [5, 10]. Clinical trials showed ambiguous results with respect to hormonal therapy effectiveness in RCC management. Many studies stated that there is no statically significant difference in OS or DFP comparing to other treatment modalities. Considering its side effects profile, hormonal therapy is not commonly used as treatment modality in RCC [10].

5. Immunotherapy

5.1 Cytokine therapy

Traditional immunotherapy initially consisted of interferon alpha (INF- α), and then high dose interleukin-2 was included (HD IL-2). These therapies were developed as other modalities such as radiation, chemotherapy, and hormonal therapy failed to achieve satisfactory response.

Multiple papers have studied the use of interleukin-2 (IL-2). Interferon alpha and both combined as an adjuvant therapy in RCC. Most showed that this type of adjuvant therapy does not exert any survival benefits. Some even terminated before completion due to intervention toxicity [5, 7].

A phase 3 trial assessed disease free and over-all survival in patient with stage III and IV renal cancer receiving high dose IL-2 showed that it is associated with severe toxicity in the form of CNS and hematologic toxicities. There were no meaningful clinical outcomes in DFS or OS between treatment and control groups [11].

Another paper used low dose IL-2 versus observation showed no benefit [12].

In a study compared the use of INF- α , IL-2 or combination as adjuvant, there was no change in OS with insignificant DFS rates.

A meta-analysis done on conventional adjuvant therapy stated that there is no benefit from using such treatment modalities [7].

Most studies collectively suggest that while IL-2 has been explored as an adjuvant therapy in RCC, the evidence does not support its effectiveness in improving survival. However, high dose IL-2 has been used in precisely selected patients who have ECOG score of 0–1 with metastatic clear cell RCC that failed other modalities, with preserved renal and hepatic function and can tolerate such modality [13].

5.2 Tumor vaccines

Autologous vaccines have been studied extensively knowing the sensitivity of renal cancer to immune-based therapies. They aim to provoke the immune system to identify and attack cancer cells. Tumor vaccines are made of tumor cells that act like antigens.

There are 3 main types of tumor vaccines including: Peptides, dendritic cells, and whole tumor vaccines with the aim to train the immune system to effectively kill all tumor cells.

The advantage of using this modality is its limited cross sensitivity to healthy tissue with the formation of specific immunity to tumor cells, confined pre-formed tumor antigen tolerance, and the capability to create a broad response to numerous tumor antigens restricting its resistance [7, 14].

Two randomized trials tested bacillus Calmette-Guerin mixed with irradiated autologous tumor cells as adjuvant in RC. Results showed no difference in prolonged DFS [15, 16].

Moreover, autologous heat shock proteins that are tumor derived (glycoprotein 96)- peptide complex (HSPPC-96;) found no improvement of DFS [17].

One study reported improvement in DFS only, with autologous renal tumor cell vaccine. However, results were criticized as there is an imbalance in the loss of patients who left the trial after randomization and the lack of overall survival tabulation [5, 14].

Four studies investigated the use of tumor vaccines in renal cell carcinoma and all stated a statically significant response DFS and OS was not observed [14].

In a phase 3 trial, oncopage was given to patients with high recurrence rate post nephrectomy. No difference was found in progression free survival and recurrence rates between control and observation group [14].

Tumor vaccines are being studied to date and not implemented yet in clinical practice.

5.3 Adoptive cell transfer

Adoptive cell transfer (ACT) is an immunotherapy in which immune cells are harvested either directly from the blood by isolating immune cells or by extracting tumor infiltrating lymphocytes from a tumor biopsy. Harvested immune cells get activated and expanded in vitro by using cytokines to enhance their tumor recognition abilities and to better attack tumor cells. Lastly, enhanced immune cells are re-infused into the patient. RCC is known for its ability to evade immune cells and with the limited effectiveness of traditional therapies, researchers explored various modalities including ACT. RCC bears TILs especially CD-8 and cytotoxic T lymphocytes which can potentially recognize and kill tumor cells. There are multiple techniques for the application of ACT [18]. **Table 1** summarizes almost most trials done exploring ACT.

5.4 Allogeneic hematopoietic stem cell transplantation

Allogeneic hematopoietic stem cell (HSC) transplantation, called allo-HSCT, carries promising potentials in treatment of primary and metastatic RCC through its immune graft-versus-tumor (GvT) effect. However, its application is limited due to graft-versus-host disease (GvHD) reaction which can be fatal [18]. Many trials studied this modality of treatment, most of which are summarized in Bregni et al.

Study	Participants	Intervention	Response %	TRM	Reference No
Childs et al.	19	Flu+Cy	53	12	[19]
Bregni et al.	7	Thio+flu+Cy	57	14	[20]
Pedrazzoli et al.	7	Flu+Cy	0	29	[21]
Schleypen et al.	12	Flu+Cy	33	33	[22]
Ueno et al.	15	Flu+Mel	47	22	[23]
Hentschke et al.	10	Flu+TBI	30	30	[24]
Baron et al.	7	Flu+TBI	14	0	[25]
Blaise et al.	25	ATG + Bu + Flu	8	9	[26]
Nagakawa et al.	9	ATG + Bu + Flu	11	0	[27]
Massenkeil et al.	7	ATG + Flu+Cy	29	14	[28]
Tykodi et al.	8	Flu+TBI	13	0	[29]
Reni et al.	22	Flu+Cy	0	9	[30]
Peres et al.	16	Flu+Cy + TBI	31	12	[31]
Ishiyama et al.	7	Flu+Cy + TBI	71	14	[32]

Table 1.
HSC trials.

paper. It summarized the results of 14 clinical studies, demonstrating response rates varying from 0 to 71%. Acute and chronic GvHD were observed in half of patients, with transplant-related deaths observed in 0–33% of patients [33].

6. Targeted immunotherapy

Management options for patients with RCC were limited with the mainstay intervention being surgical resection. Traditional therapy for RCC includes chemotherapy, radiotherapy, and immunotherapy. For advanced and metastatic diseases, conventional immunotherapies with IL-2 and INF- α were used with low therapeutic potentials and high side effects profiles. However, after the discovery of targeted therapies, an evolving revolution in the management of RCC has led to better outcomes and life quality for patients with RCC [5, 7, 13, 14]. Targeted immunotherapy comprises utilizing medications that precisely aim specific molecular pathways involved in cancer cells survival, growth, and metastasis. They specifically interfere with certain genetic alterations and molecules that propel the cancerous process unlike conventional therapies which target any rapidly growing cells. This accuracy is required for an improved efficacy and reduced side effects. By understanding the key pathways involved in RCC, these targeted therapies have evolved. One of the critical pathways implicated in RCC pathogenesis is Von Hippel-Lindau-Hypoxia-Inducible Factor (VHL-HIF). VHL is a protein called tumor suppressor protein which plays a crucial role in regulating the degradation of hypoxia-inducible factors (HIFs) [5, 18]. Mutation in VHL is commonly found in RCC, and it contributes to the disease progression and aggressiveness. When mutated, VHL protein losses its function resulting in the formation and accumulation of defective HIF which gets persistently

activated in hypoxic and even normal oxygen conditions and promotes cell survival. HIF serves as a transcription factor for variable pro-oncogenic genes that promotes tumor metabolism, angiogenesis, and cell cycle regulation such as VEGF. Persistent HIF activation leads to constant oncogenes activation and thus tumor formation and survival. Such pathways offer significant target to develop therapies for patients with RCC [7, 18]. It is important to understand the underlying pathways involved in RCC to understand the mechanism of action of each modality. **Table 2** summarizes most of the currently in use agents with the recent recommendations.

6.1 Vascular endothelial growth factor (VEGF) pathway

The VEGF pathway is an essential step for the growth and metastasis of RCC. VEGF is a signaling protein that is crucial for angiogenesis by stimulating endothelial

Category	Drug	Target	Notes
Monoclonal antibody	Pembrolizumab	PD-1	Combined with TKIs axitinib or lenvatinib to be used as first-line therapy for advanced ccRCC. Significantly high PFS and OS for lenvatinib+ pembrolizumab, with more serious side effects than axitinib+ pembrolizumab or cabozantinib+ nivolumab [34]. When used with lenvatinib, it has higher incidences of lymphatic system, blood, vascular and metabolism disorders, while the combination with axitinib has higher incidence of hepatobiliary and cardiac toxicity, axitinib has more easily manageable adverse events with a shorter half-life [35]
	Nivolumab		Nivolumab + cabozantinib or ipilimumab is recommended as first-line therapy. When combined with cabozantinib, it showed slightly superior OS and PFS with more serious toxicities than ipilimumab + nivolumab [34]
	Avelumab	PD-L1	Avelumab + axitinib is first-line therapy for PD-L1 positive advanced ccRCC. Compared with other anti-PD-L1 antibodies, such as durvalumab and atezolizumab, avelumab has a shorter half-life [36]
	Ipilimumab	CTLA-4	First-line in combination with Nivolumab for advanced ccRCC with poor and intermediate risk. Lower percentage of serious adverse events than other potent combinations [34].
TKI (multi-kinase)	Cabozantinib	VEGFR	Prolonged PFS for intermediate- or poor-risk advanced RCC compared with sunitinib. Used for advanced ccRCC as first-line therapy in combination with Nivolumab. When ICI is not suitable, it can be used alone [37]. A powerful TKI with inferior selectivity. When compared to axitinib or lenvatinib combined with pembrolizumab, it has a lower toxicity profile.
	Lenvatinib	VEGFR, FGFR	First-line when combined with pembrolizumab for advanced ccRCC. Very powerful TKI with low selectivity. More significant adverse effects with better PFS and OS when combined with pembrolizumab than axitinib+ pembrolizumab or cabozantinib + Nivolumab [34].

Category	Drug	Target	Notes
TKI	Sunitinib	VEGFR	Previously used as a first-line agent. Compared to majority of first-line conjunctions of ICI-ICI or ICI-TKI, it has low performance rates when used alone. Used when ICI therapy is contraindicated. Has similar OS to sorafenib with superior PFS [38].
	Pazopanib		An option when ICI therapy is not suitable. It was created as a newer, more targeted treatment. It offers improved patient-reported outcomes compared to sunitinib [39].
	Sorafenib		An alternative option when ICI therapy is not appropriate. It shows shorter PFS compared to sunitinib, but with comparable OS. It is more effective than tivozanib in terms of OS [40].
	Tivozanib		An option for patients with relapsed or refractory advanced RCC after at least two previous systemic treatments. It demonstrates better PFS and ORR, but shows lower OS compared to sorafenib [40].
	Axitinib		When used in combination with pembrolizumab or avelumab, it can be considered a first-line therapy for advanced ccRCC. Highly selective for VEGFR. Has higher OS, PFS and ORR when compared to sunitinib [41]. Shorter half-life compared to other TKIs.
Molecule inhibitors	Everolimus	mTOR	Superior to sunitinib when combined with lenvatinib, comparing PFS only without OS benefit [42] and can be considered after first-line therapies.

Table 2.
 ccRCC (*clear cell renal cell carcinoma*), CTLA-4 (*cytotoxic T-lymphocyte associated protein 4*), FGFR(*fibroblast growth factor receptor*), ICI (*immune checkpoint inhibitors*), mTOR (*mammalian target of rapamycin*), ORR (*overall response rate*), OS (*overall survival*), VEGFR(*vascular endothelial growth factor receptor*), PD-1 (*programmed cell death receptor-1*), PD-L1 (*programmed cell death ligand-1*), PFS (*progression free survival*), TKI(*tyrosine kinase inhibitor*).

cells proliferation, migration, and thus making new blood vessels. Vascular endothelial growth factor receptors (VEGFR) VEGFR-1, VEGFR-2, and VEGFR-3mediate tumor angiogenesis. By exploring this pathway, researchers developed Tyrosine kinase inhibitors (TKIs).

TKIs such as pazopanib, sunitinib, cabozantinib, and axitinib inhibit tumor growth by disturbing angiogenesis.

6.2 mTOR pathway

Dysregulation of the mammalian target of rapamycin (mTOR) pathway is common pathology in RCC.

mTOR inhibitors such as Temsirolimus and Everolimus increase cancer cells apoptosis and reduce proliferation by inhibiting this pathway.

Programmed Death-1 (PD-1) and Programmed Death-Ligand 1 (PD-L1) Pathway:

Immune checkpoint inhibitors developed to reactivate immunity by targeting PD-1 and PD-L1.

Examples include but not limited to: Pembrolizumab, Nivolumab, and Avelumab.

6.3 Monoclonal antibodies

Are molecules that bind to specific antigens on cancer cells. They mark cancer cells facilitating their destruction. Inhibit tumor growth by blocking its growth signals and direct cytotoxic agents to cancerous cells.

6.4 Immune checkpoint inhibitors

These drugs make T cells aggressively attack tumor cells. They work by blocking proteins that inhibit the immune response and by exploiting immune check points, thus enhancing detection of evading tumor cells.

Most targeted checkpoints are cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 and its ligand (PD-1/PD-L1). Examples of ICI are Nivolumab (anti-PD-1) and Pembrolizumab (anti-PD-L1).

6.5 Clinical trials

Understanding these mechanisms helped researches to increase their clinical knowledge and develop various medications to be used in adjuvant setting.

There are multiple randomized adjuvant clinical trials, involving targeted therapy agents. Few of these trials involve tyrosine kinase inhibitors, while one involves an mTOR inhibitor and the other a monoclonal chimeric antibody. So far, four of these trials have been completed including the ARISER, ASSURE, S-TRAC, and PROTECT trials while the other ones are still in progress. **Figure 1** summarizes some of these trials.

6.5.1 Checkmate914 trial

A phase 3 trial that randomized 411 patients to placebo versus 405 patients to Nivolumab plus Ipilimumab post-surgical resection. Results showed that

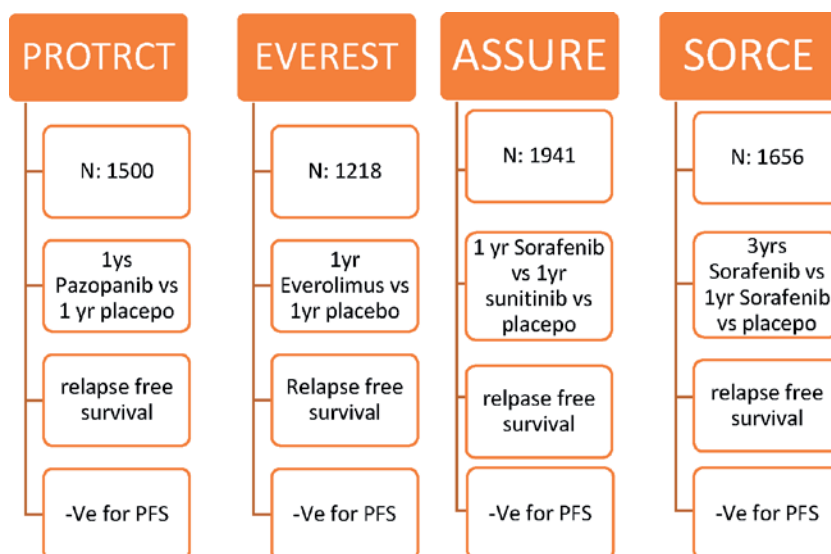


Figure 1.
Summary of four important trials done on adjuvant therapies.

Ipilimumab (anti-CTLA-4) plus Nivolumab (anti-PD-1) as adjuvant therapy did not improve DFS VS placebo in high recurrence localized renal cell carcinoma post-surgical resection [17].

6.5.2 KEYNOTE-564

Phase 3 trial evaluated using Pembrolizumab (anti-PD-1) as adjuvant therapy in 496 patients vs. placebo. DFS was the endpoint. Safety and OS were the secondary outcomes. 488 patients were treated and only 298 completed the trial. Median follow-up was 57.2 months, and OS in the placebo arm was 86.0% versus 91.2% in the Pembrolizumab arm. DFS was 56.6% and 64.9%, respectively. 32.4% of patients faced grade 3 and above adverse effects, leading to 10.0% discontinuations. KEYNOTE-564 concluded that Pembrolizumab is associated with higher OS and DS in comparison with placebo [43]. This is the first adjuvant therapy that shows actual survival benefits and is recommended as adjuvant therapy in carefully selected patients [13].

6.5.3 ADAPT

This on-going phase 3 trial randomized patients into 2 groups. 307 patients received combination Rocapuldencel-T plus standard of care (SOC) therapy and 155 he SOC only. Median OS in the SOC group was 32.4 months comparing to 27.7 in the combination group. So far, this trial concluded that using combination therapy (Rocapuldencel-T plus SOC) did not improve overall survival [44].

6.5.4 SORCE

This phase 3 trial completed on 2020. It recruited 430 patients in arm A (3 years of placebo), 642 patients in arm B (1 year of sorafenib followed by 2 yrs. of placebo), and 639 participants in arm C (3 years of sorafenib). It concluded that there was no clinically significant difference in OS nor DFS among all groups [45].

6.5.5 ARISER

864 patients were randomized to placebo (431) or Girentuximab (433). There was no difference in DFS between the two groups. OS was never reached in both arms [42, 46].

6.5.6 PROSPER

There was no clinically significant difference in recurrence free survival between patients who undergone surgery only and those who received Nivolumab. 10% of patients in the surgery group and 12% of the Nivolumab group died with 1% and 2%, respectively, are considered treatment related [47].

6.6 Risks of adjuvant therapy

Finally, balance between adverse effects of adjuvant therapy and its benefits must be achieved. Risks should not outweigh the benefits. VEGF inhibitors showed improvement in adverse events in comparison with traditional immune therapies. However, side effects are still reported in the on-going trials. Immune checkpoint

inhibitors are starting to shine and starting to have an impact in RCC management with toxicity profiles favorable to chemotherapy. They still can cause remarkable side effects. Therefore, in patients with metastatic disease, such toxicities might be tolerable. However, this is not the case in patients receiving these modalities as adjuvant therapy who in fact could have no residual cancer. Adjuvant therapy may carry risks with potential morbidity and mortality without adequate therapeutic response. Therefore, improvement in prediction criteria and selection of patients who are at high risk of recurrence.

7. Conclusion

Curative therapy for advanced RCC is still rare despite the numerous various treatment modalities and advances made in clinical trials. In the clinical practice guideline of the European Society for Medical Oncology (ESMO), the recommended first-line treatment modalities for patients with advanced ccRCC are either a combination of ICI-TKI or dual immunotherapy ICI-ICI. **Table 2** summarizes these therapies, their targets, and categories. Currently, EUA recommendation to offer adjuvant Pembrolizumab to ccRCC patients with a recurrence risk, is similar to KEYNOTE-564 trial recommendations. Treatment to be started within 12 to 16 weeks post resection is weak. There are no strong recommendations when it comes to adjuvant therapy use in locally advanced disease post resection yet despite the multiple papers done. This might be due to the conflicting data and lack of research to explore such management options. However, immunotherapy is promising as the new future for cancer treatment, and clinicians should explore such modality.

Acknowledgements

I would love to deeply thank Sumaya, Asma'a and Shahad Abdulkarem as they were huge supporters of this work.

Author details

Lujin Alajmani^{1,2*}, Badr Rafi^{1,2}, Hassan Fadil^{1,2} and Rowaa A. Banjar³

1 King Abdullah International Medical Research Center, Saudi Arabia

2 Ministry of National Guard Health Affairs, Jeddah, Saudi Arabia

3 Department of Urology, King Abdulaziz Medical City, National Guard Health Affairs, Jeddah, Saudi Arabia

*Address all correspondence to: lujin.b.aj@gmail.com

IntechOpen

© 2026 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] Bukavina L, Bensalah K, Bray F, Carlo M, Challacombe B, Karam JA, et al. Epidemiology of renal cell carcinoma: 2022 update. *European Urology*. 2022;**82**(5):529-542. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0302283822026094?via%3Dihub>
- [2] EAU Guidelines on RCC - INTRODUCTION - Uroweb [Internet]. Uroweb - European Association of Urology. Available from: <https://uroweb.org/guidelines/renal-cell-carcinoma>
- [3] Gansler T, Fedewa SA, Flanders WD, Pollack LA, Siegel DA, Jemal A. Prevalence of cigarette smoking among patients with different histologic types of kidney cancer. *Cancer Epidemiology Biomarkers & Prevention*. 2020;**29**(7):1406-1412
- [4] Lee SW, Kim HJ, Kazmi SZ, Choi YJ, Hong G, Kim YS, et al. Familial risk of renal cell cancer and interaction with obesity and hyperglycemia: A population-based study. *The Journal of Urology*. 2022;**208**(2):251-258. Available from: <https://pubmed.ncbi.nlm.nih.gov/35298291/>
- [5] Hassan F, Lambe S, Sharma K, Kapoor A. Current Role of Adjuvant Therapy in High Risk for Recurrence Resected Kidney Cancer. London, UK: IntechOpen; 2020. DOI: 10.5772/intechopen.78684
- [6] Stephenson AJ, Chetner M, Rourke K, Gleave M, Signaevsky M, Palmer B, et al. Guidelines for the surveillance of localized renal cell carcinoma based on the patterns of relapse after nephrectomy. *The Journal of Urology*. 2004;**172**(1):58-62
- [7] Lenis AT, Donin NM, Johnson DC, Faiena I, Salmasi A, Drakaki A, et al. Adjuvant therapy for high risk localized kidney cancer: Emerging evidence and future clinical trials. *Journal of Urology*. 2018;**199**(1):43-52
- [8] Kjaer M, Iversen P, Hvidt V, Bruun E, Skaarup P, Bech Hansen J, et al. A randomized trial of postoperative radiotherapy versus observation in stage II and III renal adenocarcinoma. A study by the Copenhagen renal cancer study group. *Scandinavian Journal of Urology and Nephrology*. 1987;**21**(4):285-289. Available from: <https://pubmed.ncbi.nlm.nih.gov/3445125/>
- [9] Czarnecka AM, Niedzwiedzka M, Porta C, Szczylik C. Hormone signaling pathways as treatment targets in renal cell cancer (review). *International Journal of Oncology*. 2016;**48**(6):2221-2235
- [10] Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. 1999;**399**(6733):271-275
- [11] Gandhi U, Madan RA, Aragon-Ching JB. The immunotherapy revolution in genitourinary malignancies. *Immunotherapy*. 2020;**12**(11):819-831. Available from: <https://pubmed.ncbi.nlm.nih.gov/32594815/>
- [12] Clark JI, Atkins MB, Urba WJ, Creech S, Figlin RA, Dutcher JP, et al. Adjuvant high-dose bolus Interleukin-2 for patients with high-risk renal cell carcinoma: A cytokine working group randomized trial. *Journal of Clinical Oncology*. 2003;**21**(16):3133-3140

- [13] Dibajnia P, Cardenas LM, Lalani AA. The emerging landscape of neo/adjuvant immunotherapy in renal cell carcinoma. *Human Vaccines & Immunotherapeutics*. 2023;**19**(1):2178217. DOI: 10.1080/21645515.2023.2178217
- [14] Powles T, Albiges L, Bex A, Comperat E, Grünwald V, Kanesvaran R, et al. Renal cell carcinoma: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Annals of Oncology: Official Journal of the European Society for Medical Oncology*. 2024;**35**(8):692-706. DOI: 10.1016/j.annonc.2024.05.537
- [15] Sönmez M, Sönmez L. New treatment modalities with vaccine therapy in renal cell carcinoma. *Urology Annals*. 2019;**11**(2):119
- [16] Van Poppel H, Joniau S, Van Gool SW. Vaccine therapy in patients with renal cell carcinoma. *European Urology*. 2009;**55**(6):1333-1344
- [17] Adler A, Gillon G, Lurie H, Shaham J, Loven D, Shachter Y, et al. Active specific immunotherapy of renal cell carcinoma patients: A prospective randomized study of hormone-immuno-versus hormonotherapy. Preliminary report of immunological and clinical aspects. *Journal of Biological Response Modifiers*. 1987;**6**(6):610-624. Available from: <https://pubmed.ncbi.nlm.nih.gov/3330126/>
- [18] Wang Y, Suarez ER, Kastrunes G, de Campos NSP, Abbas R, Pivetta RS, et al. Evolution of cell therapy for renal cell carcinoma. *Molecular Cancer*. 2024;**23**(1):8. DOI: 10.1186/s12943-023-01911-x
- [19] Childs RW, Chernoff A, Contentin N, Bahceci E, Schrupp DS, Leitman SF, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *The New England Journal of Medicine*. 2000;**343**(11):750-758
- [20] Bregni M, Doderio A, Peccatori J, Pescarollo A, Bernardi M, Sassi I, et al. Nonmyeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer. *Blood*. 2002;**99**(11):4234-4236
- [21] Pedrazzoli P, Da A, Giorgiani G, Schiavo B, Zambelli A, Giraldi E, et al. Allogeneic blood stem cell transplantation after a reduced-intensity, preparative regimen. *Cancer*. 2002;**94**(9):2409-2415
- [22] Schleypen JS, Geldern M v, Weiß EH, Kotzias N, Rohrmann K, Schendel DJ, et al. Renal cell carcinoma-infiltrating natural killer cells express differential repertoires of activating and inhibitory receptors and are inhibited by specific HLA class I allotypes. *International Journal of Cancer*. 2003;**106**(6):905-912
- [23] Ueno NT, Cheng YC, Rondon G, Tannir NM, Gajewski J, Couriel DR, et al. Rapid induction of complete donor chimerism by the use of a reduced-intensity conditioning regimen composed of fludarabine and melphalan in allogeneic stem cell transplantation for metastatic solid tumors. *Blood*. 2003;**102**(10):3829-3836
- [24] Hentschke P, Barkholt L, Uzunel M, Mattsson J, Wersäll P, Pisa P, et al. Low-intensity conditioning and hematopoietic stem cell transplantation in patients with renal and colon carcinoma. *Bone Marrow Transplantation*. 2003;**31**(4):253-261. Available from: <https://pubmed.ncbi.nlm.nih.gov/12621459/>
- [25] Baron F, Frère P, Baudoux E, Sautois B, Fillet G, Beguin Y.

- Non-myeloablative stem cell transplantation with low-dose total body irradiation and fludarabine for metastatic renal cell carcinoma. *Haematologica*. 2003;**88**(4):478-480. Available from: <https://pubmed.ncbi.nlm.nih.gov/12681980/>
- [26] Blaise D. Reduced-intensity preparative regimen and allogeneic stem cell transplantation for advanced solid tumors. *Blood*. 2004;**103**(2):435-441
- [27] Nakagawa T, Kami M, Hori A, Kim SW, Murashige N, Hamaki T, et al. Allogeneic hematopoietic stem cell transplantation with a reduced-intensity conditioning regimen for treatment of metastatic renal cell carcinoma: Single institution experience with a minimum 1-year follow-up. *Experimental Hematology*. 2004;**32**(7):599-606. Available from: <https://pubmed.ncbi.nlm.nih.gov/15246155/>
- [28] Massenkeil G, Roigas J, Nagy M, Wille A, Stroszczyński C, Mapara MY, et al. Nonmyeloablative stem cell transplantation in metastatic renal cell carcinoma: Delayed graft-versus-tumor effect is associated with chimerism conversion but transplantation has high toxicity. *Bone Marrow Transplantation*. 2004;**34**(4):309-316. Available from: <https://pubmed.ncbi.nlm.nih.gov/15220955/>
- [29] Tykodi SS, Fujii N, Vigneron N, Lu S, Mito JK, Miranda MX, et al. *C19orf48* Encodes a minor histocompatibility antigen recognized by CD8⁺ cytotoxic T cells from renal cell carcinoma patients. *Clinical Cancer Research*. 2008;**14**(16):5260-5269
- [30] Rini BI, Halabi S, Barrier R, Margolin KA, Avigan D, Logan T, et al. Adoptive immunotherapy by allogeneic stem cell transplantation for metastatic renal cell carcinoma: A CALGB intergroup phase II study. *Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation*. 2006;**12**(7):778-785. Available from: <https://pubmed.ncbi.nlm.nih.gov/16785067/>
- [31] Peres E, Abidi MH, Mellon-Reppen S, Klein J, Braun T, Abella E, et al. Reduced intensity transplantation for metastatic renal cell cancer with 2-year follow-up. *Journal of immunotherapy (Hagerstown, Md: 1997)*. 2007;**30**(5):562-566. Available from: <https://pubmed.ncbi.nlm.nih.gov/17589297/>
- [32] Ishiyama K, Takami A, Suzuki S, Konaka H, Namiki M, Ooi A, et al. Relationship between tumor-infiltrating T lymphocytes and clinical response after reduced-intensity allogeneic hematopoietic stem cell transplantation for advanced renal cell carcinoma: A single center prospective study. *Japanese Journal of Clinical Oncology*. 2009;**39**(12):807-812. Available from: <https://pubmed.ncbi.nlm.nih.gov/19770130/>
- [33] Bregni M, Herr W, Blaise D. Allogeneic stem cell transplantation for renal cell carcinoma. *Expert Review of Anticancer Therapy*. 2011;**11**(6):901-911
- [34] Santoni M, Rizzo A, Mollica V, Rosellini M, Marchetti A, Fragomeno B, et al. Pembrolizumab plus lenvatinib or axitinib compared to nivolumab plus ipilimumab or cabozantinib in advanced renal cell carcinoma: A number needed to treat analysis. *Expert Review of Pharmacoeconomics & Outcomes Research*. 2021;**22**(1):45-51
- [35] Matsumoto J, Iwata N, Watari S, Ushio S, Shiromizu S, Takeda T, et al. Adverse events of Axitinib plus pembrolizumab versus lenvatinib plus pembrolizumab: A pharmacovigilance

study in Food and Drug Administration adverse event reporting system. *European Urology Focus*. 2023;**9**(1):141-144. Available from: <https://pubmed.ncbi.nlm.nih.gov/35915038/>

[36] Jin H, D'Urso V, Neuteboom B, McKenna SD, Schweickhardt R, Gross AW, et al. Avelumab internalization by human circulating immune cells is mediated by both Fc gamma receptor and PD-L1 binding. *Oncoimmunology*. 2021;**10**(1):1958590. DOI: 10.1080/2162402X.2021.1958590

[37] Choueiri TK, Hessel C, Halabi S, Sanford B, Michaelson MD, Hahn O, et al. Cabozantinib versus sunitinib as initial therapy for metastatic renal cell carcinoma of intermediate or poor risk (Alliance A031203 CABOSUN randomised trial): Progression-free survival by independent review and overall survival update. *European Journal of Cancer*. 2018;**94**:115-125. Available from: <https://www.sciencedirect.com/science/article/pii/S0959804918301813>

[38] Liu XL, Xue HY, Chu Q, Liu JY, Li J. Comparative efficacy and safety of sunitinib vs sorafenib in renal cell carcinoma: A systematic review and meta-analysis. *Medicine*. 2020;**99**(13):e19570. Available from: <https://pubmed.ncbi.nlm.nih.gov/32221075/>

[39] Carducci MA. Flashback foreword: Pazopanib in renal cell carcinoma and overall survival with sunitinib versus interferon- α in metastatic renal cell carcinoma. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*. 2023;**41**(11):1955-1956. Available from: <https://pubmed.ncbi.nlm.nih.gov/37018918/>

[40] Rini BI, Pal SK, Escudier BJ, Atkins MB, Hutson TE, Porta C, et al. Tivozanib versus sorafenib in patients

with advanced renal cell carcinoma (TIVO-3): A phase 3, multicentre, randomised, controlled, open-label study. *The Lancet Oncology*. 2020;**21**(1):95-104

[41] Numakura K, Muto Y, Naito S, Hatakeyama S, Kato R, Koguchi T, et al. Outcomes of axitinib versus sunitinib as first-line therapy to patients with metastatic renal cell carcinoma in the immune-oncology era. *Cancer Medicine*. 2021;**10**(17):5839-5846. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8419787/#:~:text=The%20objective%20response%20rate%2C%20progression>

[42] Motzer R, Alekseev B, Rha SY, Porta C, Eto M, Powles T, et al. Lenvatinib plus pembrolizumab or everolimus for advanced renal cell carcinoma. *New England Journal of Medicine*. 2021;**384**(14):1289-1300

[43] Motzer RJ, Russo P, Grünwald V, Tomita Y, Zurawski B, Parikh O, et al. Adjuvant nivolumab plus ipilimumab versus placebo for localised renal cell carcinoma after nephrectomy (CheckMate 914): A double-blind, randomised, phase 3 trial. *Lancet (London, England)*. 2023;**401**(10379):821-832. DOI: 10.1016/S0140-6736(22)02574-0

[44] Powles T, Tomczak P, Park SH, Venugopal B, Ferguson T, Symeonides SN, et al. Pembrolizumab versus placebo as post-nephrectomy adjuvant therapy for clear cell renal cell carcinoma (KEYNOTE-564): 30-month follow-up analysis of a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. *The Lancet Oncology*. 2022;**23**(9):1133-1144. Available from: [https://www.thelancet.com/journals/lanonc/article/PIIS1470-2045\(22\)00487-9/fulltext](https://www.thelancet.com/journals/lanonc/article/PIIS1470-2045(22)00487-9/fulltext)

[45] Figlin RA, Tannir NM, Uzzo RG, Tykodi SS, Chen DYT, Master V, et al. Results of the ADAPT phase 3 study of Rocapuldencel-T in combination with Sunitinib as first-line therapy in patients with metastatic renal cell carcinoma. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. 2020;**26**(10):2327-2336. Available from: <https://pubmed.ncbi.nlm.nih.gov/32034074/>

[46] Chamie K, Donin NM, Klöpfer P, et al. Adjuvant weekly girentuximab following nephrectomy for high-risk renal cell carcinoma: The ARISER randomized clinical trial. *JAMA Oncology*. 2017;**3**(7):913-920. DOI: 10.1001/jamaoncol.2016.4419

[47] Allaf ME, Kim SE, Master V, McDermott DF, Harshman LC, Cole SM, et al. Perioperative nivolumab versus observation in patients with renal cell carcinoma undergoing nephrectomy (PROSPER ECOG-ACRIN EA8143): An open-label, randomised, phase 3 study. *The Lancet Oncology*. 2024;**25**(8):1038-1052. Available from: <https://pubmed.ncbi.nlm.nih.gov/38942046/>

Edited by Soumya Basu and Amit Ranjan

Urologic Cancers - Advances and Challenges in Diagnosis and Molecular Targeted Therapy offers a timely and accessible overview of one of the most rapidly evolving areas in oncology. Bringing together foundational biology and emerging clinical practice, this volume explores how molecular insights are shaping the diagnosis and treatment of cancers of the prostate, bladder, kidney, and other genitourinary organs. Across seven expert-authored chapters, the book examines aggressive disease subtypes, mechanisms of treatment resistance, and the growing role of precision-based approaches in patient care. Readers are guided through the unique biology of neuroendocrine prostate cancer, the pathways driving castration-resistant disease, and the use of advanced three-dimensional tumor models that are transforming laboratory research and therapeutic testing. The book also highlights evolving treatment strategies for renal and urothelial cancers, including the expanding use of immunotherapy and targeted drug combinations, while addressing broader challenges such as biomarker discovery, liquid biopsy development, and data-driven tools to support clinical decision-making. Designed for students, researchers, and clinicians alike, this volume serves as both a reliable reference and a source of inspiration, offering clarity, context, and direction at the intersection of molecular discovery and patient-centered care in urologic oncology.

Thomas J. FitzGerald, Oncology Series Editor

Published in London, UK

© 2026 IntechOpen
© Jezperk1auzen / iStock

IntechOpen

ISSN 3049-8864

ISBN 978-1-83634-872-6

