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Personalized Medicine

New Perspectives

Edited by Xianquan Zhan

Personalized Medicine - New Perspectives

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Contributors
Ada Carmona, Agustín Abuchaibe, Alejandra Rojo-Sánchez, David Chica-Valle, Junwen Su, Kuldeep Vinchurkar, Lisandro Pacheco-Lugo, Lisa Vorasatit, Mahdi Barazesh, Nabil Abu el Ata, Patompong Satapornpong, Pravin Admane, Sadia Ajaz, Sajad Jalili, Sheetal Mane, Shiva Mohammadi, Shoban John, Valentina Arrieta-Bravo, Xianquan Zhan, Yirys Díaz-Olmos

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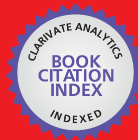

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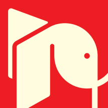

Meet the editor

Xianquan Zhan received his MD and Ph.D. in Preventive Medicine at West China University of Medical Sciences. He received his post-doctoral oncology and cancer proteomics training at Central South University in China and the University of Tennessee Health Science Center (UTHSC) in the USA. He worked at UTHSC and the Cleveland Clinic from 2001 to 2012 and achieved the rank of associate professor at UTHSC. Currently, he is a full professor at Central South University and Shandong First Medical University in China and an advisor to MS/Ph.D. students and post-doctoral fellows. He is also a fellow of the Royal Society of Medicine and European Association for Predictive Preventive Personalized Medicine (EPMA), a national representative of EPMA, and a member of the American Society of Clinical Oncology (ASCO) and the American Association for the Advancement of Sciences (AAAS). He is also editor in chief of the I*nternational Journal of Chronic Diseases & Therapy*, an associate editor of *EPMA Journal*, *Frontiers in Endocrinology*, and *BMC Medical Genomics*, and a guest editor of *Mass Spectrometry Reviews*, F*rontiers in Endocrinology*, *EPMA Journal*, and *Oxidative Medicine and Cellular Longevity*. He has published over 180 articles, 30 book chapters, 10 books, and 6 international patents in clinical proteomics and biomarkers.

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Preface

The process of human health care includes three main stages: prediction/prevention, early-stage diagnosis/therapy, and late-stage diagnosis/therapy. Individualized variations are involved in the entire healthcare process. Most diseases are multi-cause, multi-process, multi-consequence, and whole-body diseases with heterogeneity, involving a series of biological molecular changes, including DNA, RNA, protein, metabolite, etc. The conventional medical model of "the same therapy for the same disease" is being reexamined, highlighting the necessity for personalized medicine, which includes predictive care, preventive strategies, customized treatments, and individualized diagnostic and prognostic evaluations. The traditional single-parameter model must be shifted to a multi-parameter systematic model for a disease. Multiomics and systems biology are effective approaches to realize this model change. Multiomics-based signalling pathway network, pathway network-based pattern biomarkers, and then combined with clinical features and high-risk factors will deeply address the accurate molecular mechanism, stratify the patients, discover effective biomarkers, and determine the effective therapeutic targets/drugs for patients, which will significantly benefit the patients and their family to reduce the medical cost and improve the life quality of patients.

This edited volume is a collection of reviewed and relevant research chapters concerning the developments within the field of personalized medicine. The book includes scholarly contributions by various authors and is edited by a group of experts pertinent to medicine. Each contribution comes as a separate chapter, complete in itself but directly related to the book's topics and objectives.

It includes seven chapters dealing with the following topics:

1. Proteoform-Based New Perspectives for Personalized Drug Therapy

2. Translational Genetics in Hereditary Cancers for Personalized Medicine Practice

3. Novel Bio-Engineering Techniques for Construction of Next-Generation Monoclonal Antibodies in the Framework of Personalized Medicine

4. Advances in Clinical Pharmacogenomics and Prevention of Severe Cutaneous Adverse Drug Reactions in the Era of Precision Medicine

5. 3D Printing Technology in the Pharmaceutical Industry and Its Application in Drug Delivery in the Context of Personalized Medication

6. Role of Metabolomics in Precision Medicine in the Context of Systemic Lupus Erythematosus and Lupus Nephritis

7. Perspective Chapter: Making the Shift to Personalized Preventive Medicine with Human Digital Twins

These chapters present new perspectives and a comprehensive overview of recent developments in personalized medicine. This book provides a thorough overview of the latest research efforts by international authors on personalized medicine and opens new possible research paths for further novel developments.

However, this book tackles only a tiny fraction of the field of personalized medicine, which catalyzes, stimulates and encourages researchers to contribute to personalized medicine to protect human health.

The target audience comprises scholars and specialists in the field.

Xianquan Zhan, MD, PhD, FRSM
Professor of Cancer Proteomics and PPPM,
Shandong Cancer Hospital and Institute,
Medical Science and Technology Innovation Center,
Shandong First Medical University,
Shandong, Jinan, China

Chapter 1

Proteoform-Based New Perspectives for Personalized Drug Therapy

Xianquan Zhan and Junwen Su

Abstract

Personalized medicine plays important roles in the field of pharmacy because it can enhance drug efficacy, improve the treatment effects, reduce adverse reactions, and optimize treatment outcomes. Protein drugs are an important part of personalized drug therapy with higher target specificity and biological activity compared to small molecule chemical drugs. Currently, protein drugs are designed and exploited for specific protein targets according to patient-specific protein data. However, with the rapid development of proteoform concept, a canonical protein actually includes multiple proteoforms encoded by the same gene, which can lead to different responses to drugs. The effects of the different proteoforms vary considerably, which may alter the intended efficacy of the drug to potentially render it harmful rather than lifesaving. Therefore, we strongly propose to shift protein-based drugs to proteoform-based drugs, which mean that, for the development of personalized protein drugs, we should pay more attention to the differences in proteoforms encoded by the same gene to better meet the specific needs and disease characteristics. Proteoform-based personalized drug therapy will help to accurately understand the mechanisms of diseases, discover new drug targets, and provide patients with more precisely individualized treatment, which is the innovative theoretical basis to develop new drugs.

Keywords: personalized drug therapy, proteoformics, proteomics, proteoform, personalized medicine, personalized protein drugs, protein drugs, therapeutic protein drug, individualized patient profile, new perspectives

1. Introduction

1.1 Personalized medicine

Personalized medicine is a new medical concept and model, which has changed the traditional medical model [1, 2]. The core concept of personalized medicine is the use of proteomics, genomics, and other omics techniques to examine, identify, validate, and apply biomarkers in large populations of specific disease types (**Figure 1**).

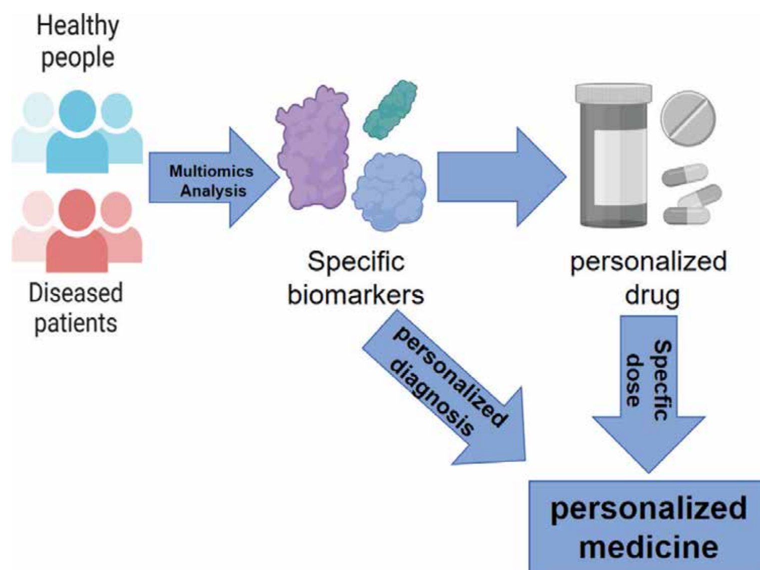

Figure 1.
Diagnostic paths of personalized medicine. Reproduced from Su et al. [3], with copyright permission from Elsevier Inc. on behalf of the American Society for Biochemistry and Molecular Biology, open access article under the CC BY license.

One can precisely identify the causes of diseases and their therapeutic targets with these omics techniques and accurately classify different disease states and processes, which can precisely stratify patients for personalized treatment and help improve the diagnosis, treatment, and prevention of certain diseases. Based on proteomics, genomics, and other omics data of each patient, medical researchers can more deeply understand the disease process, more accurately predict the patient response to drugs, and develop personalized treatment plans to improve the treatment effect and reduce unnecessary side effects and drug waste. Personalized medicine has brought great progress in the field of medicine with better treatment options and higher quality of life for patients.

1.2 Personalized drug therapy

Personalized drug therapy uses both individual genomic information (such as pharmacogenomics) and internal phenomic information (such as proteome and metabolome) to comprehensively consider each patient's genetic factors (drug metabolism gene type), gender, age, weight, physiological and pathological characteristics. And other drugs are being taken; thus, a safe, reasonable, effective, and economical drug treatment plan is formulated to achieve "individual" and "tailored" treatments. Personalized drug therapy aims to create a tailored treatment plan for each patient to maximize treatment benefits and minimize side effects. The model of personalized drug therapy covers all stages of disease prevention, analysis, diagnosis, and treatment, shifting drug delivery from symptom-based to genotype-based. The core of personalized drug therapy is to develop new individualized therapies and customize the existing drugs, taking into account the genotype, phenotype, and environmental

exposure of patients. Therefore, personalized drug therapy will improve drug efficacy, reduce adverse reactions, reduce speculation in drug use, and ultimately realize the vision of personalized medicine.

1.3 Proteoform and proteoformics

When it comes to the study on proteins, two important concepts that are closely related to protein diversity should pay attentions, namely proteoform and proteoformics.

In 2012, Smith and Kelleher first proposed the term "proteoform" to refer to the various molecular forms of a canonical protein encoded by the same gene [4]. One also should note that proteoform has two synonyms: "protein species" and "moonlighting proteins." The term "protein species" was thoroughly explained by Schluter and Jungblut [5, 6], and "moonlighting proteins" was produced by Jeffery [7]. Schluter et al. also defined the framework of the comprehensive description of an individual protein species [8]. The morphological diversity of a canonical protein is derived from multiple factors including amino acid sequence, post-translational modifications, post-transcriptional regulations, and genomic variations [4, 9]. For instance, genomic variations such as single nucleotide polymorphisms can result in multiple proteoforms. Additionally, post-transcriptional regulatory mechanisms such as post-transcriptional modifications, alternative splicing, degradation, and RNA editing all contribute to protein diversity (**Figure 2**). The advancement of two-dimensional gel electrophoresis (2DGE) and mass spectrometry (MS) has greatly improved the detection and characterization of protein diversity. Factors that contribute to a comprehensive definition of a proteoform include its binding partners, post-translational modifications, sequence of amino acids, spatial conformation, localization, presence of cofactors, and a specific function [10–12]. The term "proteoform" has been in use for 11 years since 2012 [4, 12], resulting in the emergence of the

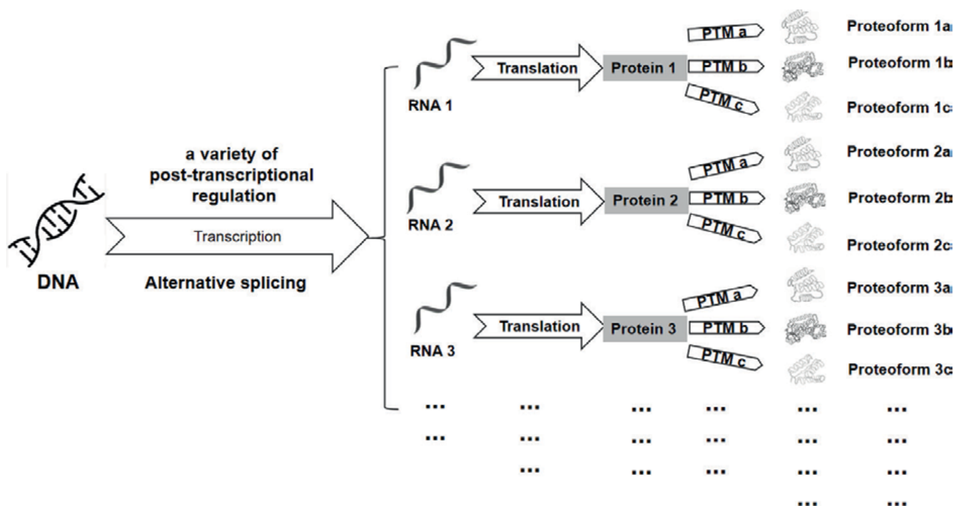

Figure 2.
The reason why the same protein has multiple proteoforms. Reproduced from Su et al. [3], with copyright permission from Elsevier Inc. on behalf of the American Society for Biochemistry and Molecular Biology open access article under the CC BY license.

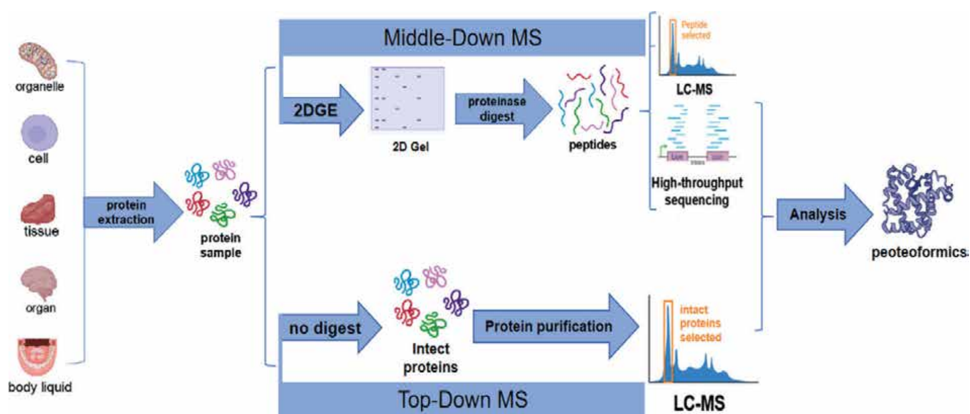

Figure 3.
The research process of proteoformics. Reproduced from Su et al. [3], with copyright permission from Elsevier Inc. on behalf of the American Society for Biochemistry and Molecular Biology, open access article under the CC BY license.

research field of proteoformics, which focuses on theories and methods for studying the morphological diversity of the proteome [9, 13].

Proteoformics is a term first proposed by Xianquan Zhan et al. in 2023, which aims to investigate the composition and dynamic changes of a canonical protein's morphological diversity within the proteoform, and the influence of this variety on physiological and pathologic processes [9, 13]. Proteoformics employs high-throughput analytical techniques and tools to distinguish the characteristics and quantitatively measure the presence and function of proteoforms (**Figure 3**). Proteoformics research procedure includes several key aspects: (i) It involves the qualification and quantification of proteoforms with high-throughput methods to determine their existence and relative abundance. (ii) It focuses on the structural analysis of proteoforms with mass spectrometry and structural biology to investigate the structure-function relationship of different proteoforms. (iii) It delves into the functional research of proteoforms to uncover their roles and interactions within cells, tissues, organs, and organisms. (iv) It hinges on data analysis with bioinformatics and computational tools to handle and decipher extensive proteoformics datasets. Proteoformics is crucial to attain a holistic comprehension of protein functionality and the mechanisms underlying diseases. The elucidation of protein diversity and regulatory processes promises significant insights. One scan enhances the understanding of the role proteins play in biological processes. This knowledge serves as a more accurate foundation for personalized medicine and drug therapy. Consequently, proteoformics research will continue to advance our understanding of proteoforms and pave the way for personalized drug research based on the use of proteoforms as targets rather than canonical proteins.

2. Meaning of personalized drug therapy

There have been significant changes in the scope of human disease with the increasing demand for drugs. Disease spectrum refers to the incidence, mortality, and composition of diseases in a specific population and a specific period, whose range is not fixed but is influenced by multiple factors such as environmental changes, lifestyle preferences, demographic characteristics, and advances in medical technology. The

main diseases that have a negative impact on people's healthy lives have evolved from ordinary infectious diseases to more complex diseases, including tumors, diabetes, and neuropsychiatric disorders [14–18]. However, the development of complex diseases is often intricate, and patient characteristics vary significantly, thus resulting in drugs that do not satisfy the sensitive population, which in turn leads to low average efficacy of drugs and a huge waste of expenditure. For example, for the same anti-cancer drug, some patients can produce positive effects, whereas other patients do not [19]. In addition, ordinary single drugs may gradually lose efficacy due to tumor mutations. Therefore, personalized drugs have important significance in the following aspects:

i) Improve therapeutic effect: Patients might differ in their different responses to a single agent. Personalized drug therapy can accurately determine the most effective drug treatment regimen per individual differences such as genotype, metabolic capacity, and drug sensitivity of patients. Personalized drug therapy considers the genetic variations of patients, their metabolic capacity, and related pathological characteristics, which can not only enhance drug efficacy but also minimize the use of ineffective drugs.

ii) Reduce adverse reactions: Patients' reactions to drugs vary, with some people being sensitive to certain drugs and others experiencing adverse reactions. Personalized drug therapy refers to predicting the sensitivity of an individual to adverse drug reactions based on the genetic information of the individual. In addition, it can reduce the likelihood of adverse effects by adjusting drug doses or selecting alternative drugs that are more suitable for individual characteristics to avoid giving patients drugs that may trigger important adverse effects.

iii) Optimize drug selection: Personalized drug therapy can combine the patient's genotype, protein expression level, metabolic capacity, and other indicators to better select drugs. It can avoid unnecessary trials, reduce the chance of treatment failure, and improve the success rate of treatment.

iv) Optimize drug selection: Patients show variable capacity of drug metabolism and excretion. Personalized drug therapy is based on the patient's metabolism, liver, and kidney function, and other factors in achieving the best effect within the therapeutic concentration range.

v) Avoid drug interactions: Some patients might take multiple medications at the same time. Personalized drug therapy can predict potential between-drug interactions by the analysis of the patient's medication list to achieve prevention goals and minimize the frequency of adverse reactions during the treatment process.

vi) Accelerate the development of new drugs: Personalized drug therapy can provide a more accurate assessment of the efficacy and safety of new drugs. Through the analysis of distinct patient subsets and consideration of individual variations within clinical trials, the efficacy of novel medications for particular demographics can be swiftly evaluated, expediting both the progression and market introduction of new pharmaceuticals.

vii) Improving the utilization of medical resources: Personalized drug therapy can improve the accuracy of drug selection and avoid ineffective treatment and trial

and error. Optimizing drug dosage and preventing adverse events can reduce medical waste and improve the efficient use of medical resources, which, in turn reduces overall healthcare costs.

viii) Promote the development of other related disciplinary fields: Personalized medicine requires the collect of multiple sets of data, including genetics, genomics, genetics, and clinical studies, which provides rich resources for precision medicine. Personalized drug therapy promotes the development of scientific research in pharmacogenetics, individual genomics, and biomarker discovery and cultivates the frontier research in the field of personalized medicine.

Therefore, personalized drug therapy plays an important role in the field of personalized medicine. It can develop a more accurate and individualized treatment plan for patients to improve the treatment effect, reduce adverse reactions, optimize drug dosage, avoid drug interactions, and minimize drug trial and error. In addition, personalized medicine can make efficient use of medical resources have significant benefits for the health and quality of life of patients, and also promote medical research and innovation. It lays a foundation for the realization of individualized and precise medical goals and will promote the development and realization of personalized medicine.

3. The relationship between personalized medicine and personalized drug therapy

Personalized medicine and personalized drug therapy are two approaches that take individual differences into account to provide more personalized and precise medical services. These approaches encompass a thorough comprehension of the patient's genetic profile, physiological state, and environmental influences. With the analysis of patients' genes, personalized drug therapy and personalized medicine can pinpoint specific genetic variations that impact their response to particular medications. Based on this information, medical staff can prescribe more effective drugs for different patients to minimize adverse reactions. In addition, these methods take into account the physiological conditions of the patient, such as organ function and metabolism. With these factors taken into account, medical service personnel can adjust treatment plans and drug doses to ensure optimal safety and efficacy. Individualized medicine also regards environmental factors that might affect a patient's health, such as lifestyle choices, toxicant exposure, and other external influences. By understanding these, medical service personnel can instruct patients how to avoid certain environmental factors or modify their lifestyle to optimize their treatment outcomes.

Personalized drug therapy is an integral part of personalized medicine, and personalized drug therapy represents the specific implementation of personalized medicine. The objective of personalized medicine is to provide tailored medical solutions with analysis of various dimensions of patient data; for example, genomics, phenotypes, and environmental factors. By considering information such as patient genotype and drug metabolism capacity, personalized drug therapy selects the most suitable treatment plan, which plays a crucial role in this process. As a result, this approach ultimately improves the effectiveness and safety of treatment. Personalized medicine takes into account the unique characteristics of each patient

to optimize their healthcare. Through genetic analysis, healthcare professionals can identify specific genetic variations that affect drug response and metabolism. This knowledge allows one to choose the most likely effective and safe drugs for patients. Additionally, personalized medicine also considers other factors, including lifestyle, environmental exposure, and comorbidities, to generate comprehensive treatment plans. Personalized medicine and drug therapy refer to the individualized treatment of patients based on their unique needs, including personalized drug selection, dosage, and duration. This is an important component of personalized medicine, and it is important to consider the genetic characteristics of patients, which may affect how drugs are metabolized and interact with the body. Medical service personnel can optimize drug therapy to maximize efficacy and minimize potential adverse reactions by considering these factors.

Therefore, personalized medication is closely related to personalized treatment. Personalized drug therapy is inseparable from personalized medicine. In addition, personalized drug therapy plays a crucial role in the field of personalized medicine. By tailoring drug therapy to the specific needs of patients, personalized drug therapy promotes the development of personalized medicine. Personalized medication and personalized treatment complement each other, working together to provide more precise medical services for different patients and ultimately improving treatment effects and enhancing patients' quality of life.

4. Disadvantages of using canonical proteins as a personalized drug target

A significant drawback of targeting drugs to canonical proteins (or proteomes) is that the same splicing protein may have multiple proteoforms. It is true that a specific protein can undergo variations, such as modifications, linkages, or other alterations, in different cell types, tissues, or disease states, to result in different functions and activities. This diversity poses some challenges for personalized drug to target proteins (or proteomes) as therapeutic targets.

i) Complexity of proteoforms: Proteoformics technologies such as two-dimensional gel electrophoresis-liquid chromatography-tandem mass spectrometry (2DE-LC/MS) and top-down mass spectrometry (TD-MS) have advanced the detection and identification of proteoforms, providing valuable insights into the complexity of the proteome and enabling the separation, identification, and analysis of different proteoforms. The complexity of proteoforms refers to the diversity and heterogeneity of canonical proteins that occur within biological systems. It is well known that different proteoforms might have distinct functions and roles [20]. These discoveries demonstrate that a canonical protein is diversified, which limits the accuracy of personalized drugs targeting a canonical protein.

ii) Target recognition and verification: The presence of multiple proteoforms in different cell types or tissues, each proteoform has its own expression levels and functions, which demonstrates that changes in individual proteoforms could potentially lead to the onset of certain diseases and result in variations in drug responses among individuals. Therefore, it is essential to conduct additional research and experimental validation on proteoforms that hold biological significance. It is also important to confirm whether specific proteoforms can be utilized as personalized drug targets instead of canonical proteins.

iii) Function analysis: The functions and regulation of proteins are usually associated with their structure and composition. Changes in proteoforms, including protein modifications, splicing variants, or mutations, might lead to alterations in protein structure and function [6, 21, 22]. It is insufficient to identify different proteoforms through proteoformics analysis. Additional experimental research is required to comprehend the roles and regulatory networks of various proteoforms. This might require the expression and purification of specific proteoforms followed by functional analysis. With cell or animal models, ones can explore the roles of specific proteoforms in cellular functions, signal transduction, and diseases. In summary, distinct proteoforms could potentially possess unique functionalities and regulatory processes. To comprehend the functions and regulatory networks of different proteoforms, more experiments and functional analyses are needed, rather than just identification at the proteomic level. Furthermore, the roles of these proteoforms in specific diseases should also be investigated.

iv) Different proteinforms can be used to develop new personalized drug targets, because potential differences in structure and function of proteoforms can lead to varied responses to drugs. If a drug targets canonical proteins that exist in multiple proteoforms, the drug might be effective on some proteoforms but ineffective on others. This inconsistency can result in adverse effects in some cases and lack of desired therapeutic effects in others, leading to safety issues [23–25]. Therefore, the development of personalized medicine based on canonical protein requires a thorough understanding of the various forms of the target protein and consideration of their potential variability. This might entail employing meticulous techniques or investigative methodologies to discern and delineate various proteoforms, along with assessing their expression patterns and functionalities in particular ailments or physiological conditions. Additionally, it is necessary to conduct experiments and clinical studies to clarify the effects of drugs on different proteoforms to ensure the safety and efficacy of personalized medicine. In conclusion, for the development of personalized medicine based on canonical protein, it is crucial to consider the variability of multiple proteoforms. Drugs targeting canonical protein might have unnecessary or harmful effects on various proteoforms, leading to safety issues and adverse effects.

v) Drug delivery and targeting: First, different proteoforms have varying locations within the cell. Certain protein types may be localized to different subcellular structures or cell membranes, requiring drugs to precisely target each individual proteoforms to exert their effects. Second, different proteoforms may exhibit differences in expression levels and persistence. Specific proteoforms may be highly unstable or expressed at lower levels within the cell, limiting the effective delivery and action of drugs. When designing drug delivery systems, these variations need to be taken into consideration. Additionally, targeting specific proteoforms poses challenges due to their specificity and subtle differences. It is essential to identify structural features or specific markers of proteoforms to aid in targeted drug delivery. Overall, proteoforms exhibit differences in cellular location, expression levels, and stability, which make them challenge targets for drug delivery compared to canonical proteins.

Therefore, the exploration of canonical proteins as therapeutic targets encounters intricacies and obstacles arising from the plethora of proteoforms. It is essential to develop personalized drugs based on proteoformics.

5. Current status of personalized drugs targeting proteoforms

Although a canonical protein has different proteoforms was not previously understood, previous examples of proteoform efficacy differences have been observed in the drug development process [3]. A prominent example is the development of Viagra, which targets the activity of phosphodiesterase (PDE) to increase cGMP levels, which reduces intracellular calcium ion concentration to dilate blood vessels. Its smooth muscle and hypotensive effects make it a novel antihypertensive and anti-angina medication. Interestingly, PDE exists in multiple proteoforms, and Viagra acts on the cyclic guanosine monophosphate-specific phosphodiesterase type 5 (PDE5) widely distributed in the penile corpus cavernosum, rather than the non-specific cyclic guanosine monophosphate found in vascular smooth muscle as expected. Viagra lacks the anticipated hypotensive and anti-angina effects but has been recognized as a specific treatment for erectile dysfunction (ED). Due to the impact of different proteoforms on drug efficacy, a drug may exhibit unexpected pharmacological effects, as seen with Viagra's efficacy in treating ED, which is undoubtedly acceptable [3]. However, it is essential for one to consider the potential safety issues if a drug not only fails to produce the expected therapeutic effects due to proteoform differences but also deteriorates the patient's condition or causes other toxic side effects (**Figure 4**). This underscores the importance for individuals to thoroughly comprehend that during drug design, careful consideration must be given to whether the drugs being developed and their targeted drug receptors encompass diverse proteoforms, and the potential ramifications that variations in these proteoforms might pose on drug effectiveness.

When one designs pharmaceuticals, the key factor in finding drug targets is the receptors in the body. Among them, G protein-coupled receptors are currently a hot research topic. However, most G protein-coupled receptors exist in different

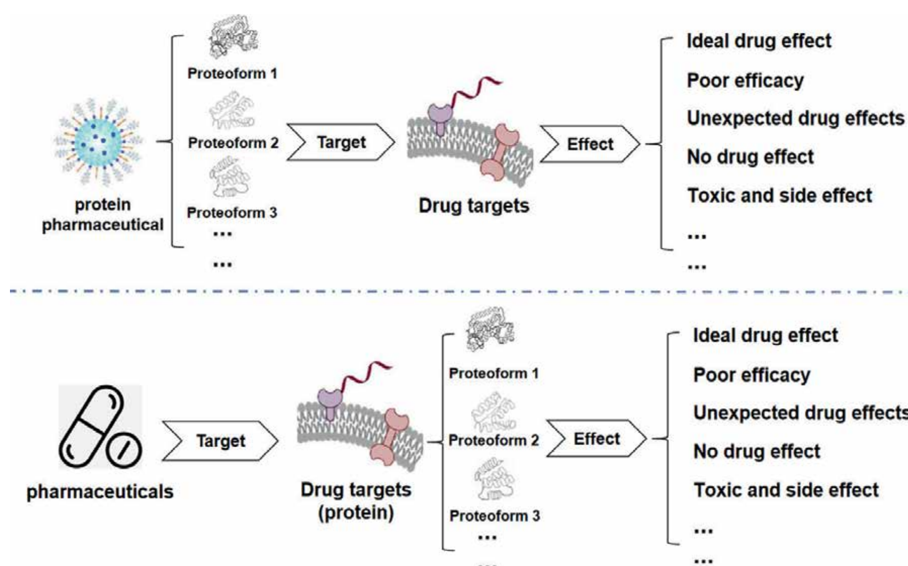

Figure 4.
Effect of proteoform on drug efficacy. Reproduced from Su et al. [3], with copyright permission from Elsevier Inc. on behalf of the American Society for Biochemistry and Molecular Biology, open access article under the CC BY license.

Effector		Receptor of efferent nervous system					
		Adrenoceptor			Cholinoceptor		
		Proteoform	Effect	Ref.	Proteoform	Effect	Ref.
Eye	Pupil opening muscles	α_1	Contract (delate pupils)	[26]			
	Pupil sphincter				M_3	Contract (miosis)	[27]
	Ciliary muscle	β_2	Slack (farsightedness)	[26]	M_3	Contract (shortsighted)	[28]
Heart	Sinus node	β_1	Increased autorhythmicity and accelerated heart rate	[29]	M_2	Decreased autorhythmicity and slowed heart rate	[30]
	Room knot	β_1	Speed up conduction	[31]	M_2	Slow down conduction	[32]
	Conduction system	β_1	Speed up conduction	[33]	M_2	Slow down conduction	[34]
	Myocardium	β_1	Increased contraction	[35]	M_2	Contraction weakened	[36]
Vascular smooth muscle	Skin, Mucosa	α_1, α_2	Contract	[37]			
	Abdominal viscera	α_1, β_2	Contract, Relax	[38]			
	Coronary blood vessels	α_1, β_2	Contract, Relax	[39]			
	Skeletal muscle	α_1, β_2	Contract, Relax	[40]	M_3	Relax	[41]
	Brain	α_1	Contract	[42]			
	Kidney	α_1, β_2	Contract, Relax	[43]			
	Lung	α_1, β_2	Contract, Relax	[44]			
Endothelium					M_3	Releases NO	[45]
Bronchial smooth muscle		β_2	Relax	[46]	M_3	Contract	[47]
Stomach and intestines	Smooth muscle	α_2, β_2	Slack	[48]	M_3	Contract	[49]
	Sphincter	α_1	Contract	[50]	M_3	Slack	[51]
Gallbladder and biliary smooth muscle		β_2	Relax	[52]	M	Contract	[53]
Bladder	Uroid muscle	β_2	Slack	[54]	M_3	Contract	[55]
	Sphincter	α_1	Contract	[56]	M_3	Slack	[55]

Effector		Receptor of efferent nervous system					
		Adrenoceptor			Cholinoceptor		
		Proteoform	Effect	Ref.	Proteoform	Effect	Ref.
Uterine smooth muscle		α_1, β_2	Contract, Slack	[57]	M_3	Contract	[58]
Salivary gland		α_1	Secrete K^+ and water	[59]	M_3	Secrete K^+ and water	[60]
		β	Secret salivary amylase	[59]			
Bronchial gland		α_1, β_2	Decreased secretion, Increased secretion	[61]	M_3	Increased secretion	[62]
Acid gland (Wall cells)					M_1	Increased secretion	[63]
Skin sweat glands		α_1	Local secretion (Palms of hands and feet)	[64]	M_3	Secrete	[65]
Paranephrocyte		β_1	Secrete renin	[66]			
Adrenal medullary					N_M	Secrete	[66, 67]
Metabolism	Hepatic gluconeogenesis	β_2, α_1	Increase	[68]			
	Hepatic glycogen decomposition	β_2, α_1	Increase	[69]			
	Fat decomposition	β_3	Increase	[70]			
Skeletal muscle		β_2	Break down glycogen	[71]	N_M	Contract	[72]

Table 1.
Different proteoforms of receptors from efferent nervous system and their different effects [3]. Reproduced from Su et al. [3], with copyright permission from Elsevier Inc. on behalf of American Society for Biochemistry and Molecular Biology open access article under the CC BY license.

proteoforms. For example, receptors in the peripheral nervous system are currently a deeply researched subject. Receptors in the peripheral nervous system react to neurotransmitters selectively and are mainly divided into cholinergic receptors and adrenergic receptors. These two types of receptors are further divided into M receptors, N receptors, alpha receptors, and beta receptors. Due to genetic encoding differences, these receptors exist in different proteoforms. Previous studies have confirmed that different proteoforms are distributed in different regions of the human body, leading to different effects (**Table 1**). This is not a random event but a common feature of several different drug targets in the human body. Despite being the same protein, due to differences in genetic encoding and other factors, they exhibit different proteoforms to result in different effects [3]. When one designs drugs and selects the intended target protein, the influence of the existing proteoforms must be considered [3]. One should, or must, target the drug to the specific proteoform one designs, rather than just target a canonical protein [3]. This will help avoid variations in drug efficacy caused by differences in proteoforms [3].

6. New development of personalized drugs under proteoformics

The study of distinct proteoforms originating from a canonical protein, known as proteoformics, plays a vital role in the advancement of personalized medicine. Proteins are the foundation of many functional molecules in organisms, and variations in their structure and function are important factors in disease occurrence and drug responses. Drug design usually relies on the structure and function of specific proteins, which are considered to have stable structural and functional characteristics. Recent studies show that each canonical protein may have numerous proteoforms [3]. Various factors, including protein modifications, protein isoforms, and protein appendages, can lead to these variations. Therefore, when dealing with proteins that have multiple proteoforms, it is essential to consider the structural and functional differences between the various proteoforms derived from a canonical protein [3]. This ensures the effectiveness of drugs against specific proteoforms. The latest advancements in proteoformics enable researchers to comprehensively understand each proteoform derived from a canonical protein, contributing to the development of personalized drug targets [3]. In particular, the new progress in proteoformics for tailored drug development encompasses the following essential aspects (**Figure 5**):

i) Research on individual differences: Proteoformics may help researchers understand the proteoform differences derived from a canonical protein. Proteoformics techniques can detect post-translational modifications of diverse proteoforms, including methylation, acetylation, ubiquitination, phosphorylation, nitration, and nitrosylation. With this personalized data, physicians can develop individualized treatment plans based on the patient's proteoformic profile. As a result, drug therapy can more precisely target the disease mechanisms in patients to improve treatment efficacy and reduce adverse reactions. In conclusion, proteoformics offers the potential to create personalized medicine based on researchers' understanding of proteoform differences between individuals. Expected outcomes include more precise and effective methods to predict, diagnose, and treat diseases.

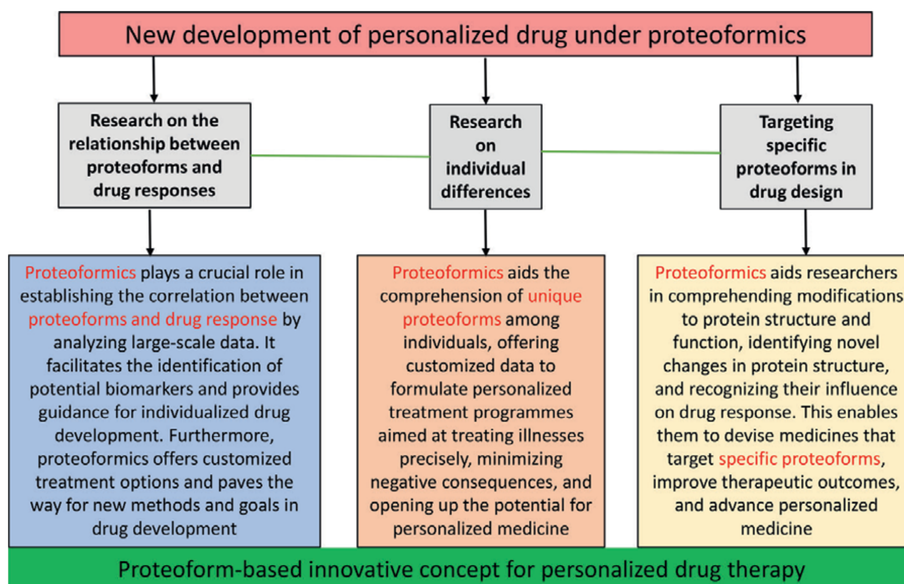

Figure 5.
New development of personalized drug under proteoformics. Modified from Su et al. [3], with copyright permission from Elsevier Inc. on behalf of the American Society for Biochemistry and Molecular Biology, open access article under the CC BY license.

ii) In drug design, targeting specific proteoforms can be facilitated by the application of proteoformics, which can assist researchers in comprehending alterations in protein structure and function. By utilizing proteoformics, researchers are able to identify and detect various changes in protein structures, including protein isoforms, mutants, or modified variants. These structural variations can significantly impact the function of proteins. Understanding specific changes in protein structure and their effects on drug responses can help in designing drugs that target precise proteoforms. For example, specific modifications to proteins can either enhance or weaken the interactions between these proteins and drugs. By leveraging this understanding, scientists can develop drugs tailored to specific protein modifications, thereby increasing drug effectiveness and reducing potential negative effects. Therefore, proteoformics can assist researchers in gaining a more detailed understanding of the internal structure and functional differences of a canonical protein. This information can be used to design drugs targeting specific proteoforms. These applications have the potential to boost the accuracy and effectiveness of drugs, thereby enhancing their overall performance to accelerate the development of personalized medicine.

iii) An exploration into the linkage between proteoforms and drug reactions: Proteoformics offers researchers the means to establish this connection and guide the advancement of personalized drug therapy. By analyzing large-scale proteoformics data, researchers can identify and quantify the correlation between specific proteoforms and drug responses. These proteoforms of a canonical protein may take the form of genetic variations, post-transcriptional modifications, or other changes in protein structure and function. Researchers can link drug response data (such as drug efficacy and adverse reactions) with

proteoformics data to identify proteoforms associated with drug responses. This process helps identify potential biomarkers, which serve as indicators of different proteoforms patterns predicting drug responses. The results of these correlation analyses can provide essential guidance for the development of personalized medicine. Based on the relationship between specific proteoforms and drug responses, researchers can design customized treatment plans for different patient populations. It may involve optimizing drug dosages, selecting appropriate drug combinations, and even customizing specific drugs to intervene with specific proteoforms. Furthermore, by establishing the correlation between proteoformic data and drug responses, researchers can provide new targets and methods for drug discovery and development. Proteoformic data can reveal new protein variations or modifications related to drug responses, inspiring new drug development targets. Proteoformics can help researchers establish the correlation between proteoformics and drug responses, which provides crucial guidance for the development of individualized drug development and enhances innovation in drug discovery and development.

Proteoformics provides comprehensive and detailed information about proteins, which contributes to the development of personalized medicine. It enhances researchers' understanding of protein functions and variations to provide more accurate guidance for personalized drug therapy, which, in turn, promotes individualized drug treatment, and improve the effectiveness and safety of drugs.

7. Conclusion

Traditional drug development and treatment methods are usually based on inter-group average effect design and promotion of drugs, which thereby ignore individual differences. However, the personal characteristics of each individual, such as genetics, physiological conditions, environment, and lifestyle, can significantly affect drug responses. Therefore, the medical field has accorded widespread attention to the concept of personalized medicine. Personalized drug therapy, as an important component of personalized medicine, is a key means to promote the development of personalized medicine. Significant progress has been made in personalized medicine and drug therapy. By combining advanced technologies with traditional experiences, damage can be reduced and precision maintained. Advances in human genetics and genomics, gene sequencing technologies, signal pathway research, gene interactions and networks, molecular regulation, and other biotechnologies will drive the future development of personalized medicine. The implementation of personalized drug therapy holds the potential to establish personalized medicine as the prevailing medical paradigm in the future, with the personalized approach to drug development emerging as the international benchmark and apex of biomedical technology.

For the advancement of personalized medicine, we strongly recommend research focusing on proteoforms as the target of personalized medicine. With the progress of proteomics methodology such as two-dimensional gel electrophoresis combined with mass spectrometry (2DE-MS) and top-down mass spectrometry (TD-MS), we have realized the impact of proteoforms on individual clinical differences. Furthermore, we strongly believe that personalized treatment targeting proteoforms is crucial for Predictive, Preventive, and Personalized Medicine (PPPM; 3P medicine) and precision medicine, with three key implications: First, in-depth study of the specificity of

proteoforms can enhance the understanding of disease mechanisms, reveal pathophysiological processes, identify new drug targets and signaling pathways, and provide theoretical basis for drug development [73, 74]. Second, detecting individual patients' proteoform levels can tailor treatment strategies. Tailoring drug targets and treatment methods based on individual differences in proteoforms can improve treatment outcomes, reduce side effects, assist physicians in health risk assessment, and make more cost-effective targeted prevention decisions [75, 76]. Additionally, proteoformics can facilitate better implementation of 3P medicine through artificial intelligence/machine learning, promote technological innovation, and provide more convenient treatment for personalized patients [77–79]. Proteoforms as drug targets have the potential to stimulate innovation in drug development. Traditional drug development methods have mainly focused on the amino acid sequences of protein targets and overlook the importance of protein conformation and modifications. With the development of personalized medicine, proteoformics will contribute to the discovery of a new generation of targeted drugs. In conclusion, the use of proteoforms as drug targets in personalized medicine creates new opportunities and challenges for PPPM and precision medicine. This approach can enhance the study and understanding of canonical proteins, improve the precision and effectiveness of drug therapy, inspire innovation in drug development, and provide more options for personalized treatment.

In summary, personalized drug therapy, as an essential component of personalized medicine, is considered a leading clinical treatment method in the future. It offers a more precise, efficient, and safe option for treatment. By advancing proteoformics research, we can better study individual differences and gain a clearer understanding of the relationship between proteins and diseases. This underscores the design and development of drugs tailored to specific proteoforms, further deepening our comprehension of individual variations in drug responsiveness and enabling the creation of tailored treatment plans for each patient. The use of proteoform as drug targets for customized drug development can refine PPPM and advance personalized medicine. The individual proteoformics related to drug responses can facilitate customized drug design, improve treatment outcomes, and provide more accurate treatment methods for individuals. This type of personalized medicine is expected to usher in a new era for PPPM and precision medicine and promote more precise, effective, and less side-effect-prone individualized treatments for various diseases.

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Author's contributions

J.S. was responsible for formal analysis, visualization, and writing-original draft; X. Z. was responsible for conceptualization, methodology, project administration, funding acquisition, writing-review and editing, and critically revision.

Conflict of interest

We declare that the authors do not have competing interests.

Author details

Xianquan Zhan* and Junwen Su
Shandong Provincial Key Laboratory of Precision Oncology, Shandong Provincial Key Medical and Health Laboratory of Ovarian Cancer Multiomics, and Jinan Key Laboratory of Cancer Multiomics, Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Jinan, Shandong, China

*Address all correspondence to: yjzhan2011@gmail.com

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References

[1] Ginsburg GS, McCarthy JJ. Personalized medicine: Revolutionizing drug discovery and patient care. Trends in Biotechnology. 2001;**19**(12):491-496. DOI: 10.1016/s0167-7799(01)01814-5

[2] Jain KK. Personalized medicine. Current Opinion in Molecular Therapeutics. 2002;**4**(6):548-558

[3] Su J, Yang L, Sun Z, Zhan X. Personalized drug therapy: Innovative concept guided with proteoformics. Molecular and Cellular Proteomics. 2024;**23**(3):100737. DOI: 10.1016/j.mcpro.2024.100737

[4] Smith LM, Kelleher NL. Consortium for top down proteomics. Proteoform: A single term describing protein complexity. Nature Methods. 2013;**10**(3):186-187. DOI: 10.1038/nmeth.2369

[5] Jungblut PR, Holzhütter HG, Apweiler R, Schlüter H. The speciation of the proteome. Chemistry Central Journal. 2008;**2**:16. DOI: 10.1186/1752-153X-2-16

[6] Schlüter H, Apweiler R, Holzhütter HG, Jungblut PR. Finding one's way in proteomics: A protein species nomenclature. Chemistry Central Journal. 2009;**3**:11. DOI: 10.1186/1752-153X-3-11

[7] Jeffery CJ. Moonlighting proteins. Trends in Biochemical Sciences. 1999;**24**(1):8-11. DOI: 10.1016/s0968-0004(98)01335-8

[8] Schlüter H, Riedner M, Jungblut PR. Protein species—The future challenge for enzymology. In: Beilstein Proceedings. ESCEC, September 23rd-26th, 2008. Rüdesheim/Rhein, Germany: Semantic Scholar; 2008. pp. 123-136. ISBN 978-3-8325-2038-0. Available from: https://api.semanticscholar.org/CorpusID:210878614

[9] Yang L, Li C, Song T, Zhan X. Growth hormone proteoformics atlas created to promote predictive, preventive, and personalized approach in overall management of pituitary neuroendocrine tumors. The EPMA Journal. 2023;**14**(3):443-456. DOI: 10.1007/s13167-023-00329-1

[10] Zhan X, Li B, Zhan X, Schlüter H, Jungblut PR, Coorssen JR. Innovating the concept and practice of two-dimensional gel electrophoresis in the analysis of proteomes at the proteoform level. Proteomes. 2019;**7**(4):36. DOI: 10.3390/proteomes7040036

[11] Zhan X, Li N, Zhan X, Qian S. Revival of 2DE-LC/MS in proteomics and its potential for large-scale study of human proteoforms. Med One. 2018;**3**:e180008. DOI: 10.20900/mo.20180008

[12] Li N, Desiderio DM, Zhan X. The use of mass spectrometry in a proteome-centered multiomics study of human pituitary adenomas. Mass Spectrometry Reviews. 2022;**41**(6):964-1013. DOI: 10.1002/mas.21710

[13] Zhan X, Su J, Yang L. Editorial: Biomolecular modifications in endocrine-related cancers. Frontiers in Endocrinology (Lausanne). 2023;**14**:1133629. DOI: 10.3389/fendo.2023.1133629

[14] Grosser T, Ricciotti E, FitzGerald GA. The cardiovascular pharmacology of nonsteroidal anti-inflammatory drugs. Trends in Pharmacological Sciences.

2017;**38**(8):733-748. DOI: 10.1016/j.tips.2017.05.008

[15] Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. Nature Reviews. Endocrinology. 2018;**14**(2):88-98. DOI: 10.1038/nrendo.2017.151

[16] Frérot M, Lefebvre A, Aho S, Callier P, Astruc K, Aho Glélé LS. What is epidemiology? Changing definitions of epidemiology 1978-2017. PLoS One. 2018;**13**(12):e0208442. DOI: 10.1371/journal.pone.0208442

[17] Relton CL, Davey SG. Epigenetic epidemiology of common complex disease: Prospects for prediction, prevention, and treatment. PLoS Medicine. 2010;**7**(10):e1000356. DOI: 10.1371/journal.pmed.1000356

[18] Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. European Journal of Cancer. 2018;**103**:356-387. DOI: 10.1016/j.ejca.2018.07.005

[19] Wu F, Wang L, Zhou C. Lung cancer in China: Current and prospect. Current Opinion in Oncology. 2021;**33**(1):40-46. DOI: 10.1097/CCO.0000000000000703

[20] Carbonara K, Andonovski M, Coorssen JR. Proteomes are of proteoforms: Embracing the complexity. Proteomes. 2021;**9**(3):38. DOI: 10.3390/proteomes9030038

[21] Jungblut PR, Thiede B, Schlüter H. Towards deciphering proteomes via the proteoform, protein speciation, moonlighting and protein code concepts. Journal of Proteomics. 2016;**134**:1-4. DOI: 10.1016/j.jprot.2016.01.012

[22] Gorr TA, Vogel J. Western blotting revisited: Critical perusal of underappreciated technical issues. Proteomics. Clinical Applications. 2015;**9**(3-4):396-405. DOI: 10.1002/prca.201400118

[23] Anjo SI, Santa C, Manadas B. SWATH-MS as a tool for biomarker discovery: From basic research to clinical applications. Proteomics. 2017;**17**(3-4). DOI: 10.1002/pmic.201600278

[24] Issaq HJ, Veenstra TD. The role of electrophoresis in disease biomarker discovery. Electrophoresis. 2007;**28**(12):1980-1988. DOI: 10.1002/elps.200600834

[25] Xu H, Wang Y, Lin S, Deng W, Peng D, Cui Q, et al. PTMD: A database of human disease-associated post-translational modifications. Genomics, Proteomics and Bioinformatics. 2018;**16**(4):244-251. DOI: 10.1016/j.gpb.2018.06.004

[26] Schwinn DA, Afshari NA. Alpha(1)-adrenergic receptor antagonists and the iris: New mechanistic insights into floppy iris syndrome. Survey of Ophthalmology. 2006;**51**(5):501-512. DOI: 10.1016/j.survophthal.2006.06.011

[27] Smith SA, Smith SE. Subsensitivity to cholinoceptor stimulation of the human iris sphincter in situ following acute and chronic administration of cholinomimetic miotic drugs. British Journal of Pharmacology. 1980;**69**(3):513-518. DOI: 10.1111/j.1476-5381.1980.tb07042.x

[28] Alm A, Nilsson SF. Uveoscleral outflow--a review. Experimental Eye Research. 2009;**88**(4):760-768. DOI: 10.1016/j.exer.2008.12.012

[29] McDevitt DG. In vivo studies on the function of cardiac beta-adrenoceptors in man. European Heart Journal.

1989;**10**(Suppl. B):22-28. DOI: 10.1093/eurheartj/10.suppl_b.22

[30] Karagueuzian HS. How does cholinergic activation slow down sinus node automaticity? “diastolic voltage oscillations” vs. “calcium clock” mechanisms. Journal of Cardiovascular Electrophysiology. 2011;**22**(1):81-83. DOI: 10.1111/j.1540-8167.2010.01866.x

[31] Frishman WH. Circulatory and metabolic aspects of beta-adrenoceptor blockade. American Heart Journal. 1988;**116**(5 Pt 2):1393-1400. DOI: 10.1016/0002-8703(88)90129-9

[32] Higgins CB, Vatner SF, Braunwald E. Parasympathetic control of the heart. Pharmacological Reviews. 1973;**25**(1):119-155

[33] Stöckigt F, Brixius K, Lickfett L, Andrié R, Linhart M, Nickenig G, et al. Total beta-adrenoceptor knockout slows conduction and reduces inducible arrhythmias in the mouse heart. PLoS One. 2012;**7**(11):e49203. DOI: 10.1371/journal.pone.0049203

[34] Halder N, Lal G. Cholinergic system and its therapeutic importance in inflammation and autoimmunity. Frontiers in Immunology. 2021;**15**(12):660342. DOI: 10.3389/fimmu.2021.660342

[35] Wallukat G. The beta-adrenergic receptors. Herz. 2002;**27**(7):683-690. DOI: 10.1007/s00059-002-2434-z

[36] Kilter H, Lenz O, La Rosée K, Flesch M, Schwinger RH, Mädge M, et al. Evidence against a role of nitric oxide in the indirect negative inotropic-effect of M-cholinoceptor stimulation in human ventricular myocardium. Naunyn-Schmiedeberg's Archives of Pharmacology. 1995;**352**(3):308-312. DOI: 10.1007/BF00168562

[37] Brown MJ. Investigation of alpha 2-adrenoceptors in humans. The American Journal of Medicine. 1989;**87**(3C):6S-9S. DOI: 10.1016/0002-9343(89)90497-x

[38] McVary KT, McKenna KE, Lee C. Prostate innervation. The Prostate. Supplement. 1998;**8**:2-13

[39] Harrison DG, Sellke FW, Quillen JE. Neurohumoral regulation of coronary collateral vasomotor tone. Basic Research in Cardiology. 1990;**85**(Suppl. 1):121-129. DOI: 10.1007/978-3-662-11038-6_10

[40] Shiuchi T, Toda C, Okamoto S, Coutinho EA, Saito K, Miura S, et al. Induction of glucose uptake in skeletal muscle by central leptin is mediated by muscle β2-adrenergic receptor but not by AMPK. Scientific Reports. 2017;**7**(1):15141. DOI: 10.1038/s41598-017-15548-6

[41] Rodrigues ACZ, Messi ML, Wang ZM, Abba MC, Pereyra A, Birbrair A, et al. The sympathetic nervous system regulates skeletal muscle motor innervation and acetylcholine receptor stability. Acta Physiologica (Oxford, England). 2019;**225**(3):e13195. DOI: 10.1111/apha.13195

[42] Rangaraj N, Kalant H, Beaugé F. Alpha 1-adrenergic receptor involvement in norepinephrine-ethanol inhibition of rat brain Na+ -K+ ATPase and in ethanol tolerance. Canadian Journal of Physiology and Pharmacology. 1985;**63**(9):1075-1079. DOI: 10.1139/y85-176

[43] Kamiar A, Yousefi K, Dunkley JC, Webster KA, Shehadeh LA. β2-adrenergic receptor agonism as a therapeutic strategy for kidney disease. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2021;**320**(5):R575-R587. DOI: 10.1152/ajpregu.00287.2020

[44] Nakamura A, Imaizumi A, YanagawaY.Beta2-AdrenoceptorFunction in the Kidney. Nihon Yakurigaku Zasshi. 2004;**124**(6):427-434. Japanese. DOI: 10.1254/fpj.124.427

[45] Whitehead AK, Erwin AP, Yue X. Nicotine and vascular dysfunction. Acta Physiologica (Oxford, England). 2021;**231**(4):e13631. DOI: 10.1111/apha.13631

[46] Lulich KM, Goldie RG, Paterson JW. Beta-adrenoceptor function in asthmatic bronchial smooth muscle. General Pharmacology. 1988;**19**(3):307-311. DOI: 10.1016/0306-3623(88)90021-3

[47] Hvizdos KM, Goa KL. Tiotropium bromide. Drugs. 2002;**62**(8):1195-1203; discussion 1204-5. DOI: 10.2165/00003495-200262080-00008

[48] Ahlman H, Nilsson O, Wängberg B, Dahlström A. Neuroendocrine insights from the laboratory to the clinic. American Journal of Surgery. 1996;**172**(1):61-67. DOI: 10.1016/S0002-9610(96)00078-5

[49] Niederberger MD, Hirsbrunner G, Steiner A, Brechbühl M, Meylan M. In vitro effects of bethanechol on abomasal and duodenal smooth muscle preparations from dairy cows with left displacement of the abomasum and from healthy dairy cows. Veterinary Journal. 2010;**184**(1):88-94. DOI: 10.1016/j.tvjl.2009.01.018

[50] Gomes LTDC, de Sena MO, Dantas PB, Barbosa AIDS, Holanda VAD, Oliveira JIN, et al. Smooth muscle contraction of the fundus of stomach, duodenum and bladder from mice exposed to a stress-based model of depression. Physiology and Behavior. 2023;**1**(272):114374. DOI: 10.1016/j.physbeh.2023.114374

[51] Lundgren O. Vagal control of the motor functions of the lower esophageal sphincter and the stomach. Journal of the Autonomic Nervous System. 1983;**9**(1):185-197. DOI: 10.1016/0165-1838(83)90140-6

[52] Lipworth BJ. Clinical pharmacology of beta 3-adrenoceptors. British Journal of Clinical Pharmacology. 1996;**42**(3):291-300. DOI: 10.1046/j.1365-2125.1996.04222.x

[53] Lee MC, Yang YC, Chen YC, Huang SC. Muscarinic receptor M3 mediates human gallbladder contraction through voltage-gated Ca2+ channels and rho kinase. Scandinavian Journal of Gastroenterology. 2013;**48**(2):205-212. DOI: 10.3109/00365521.2012.741615

[54] Michel MC, Vrydag W. Alpha1-, alpha2- and beta-adrenoceptors in the urinary bladder, urethra and prostate. British Journal of Pharmacology. 2006;**147**(Suppl. 2):S88-S119. DOI: 10.1038/sj.bjp.0706619

[55] Yamanishi T, Chapple CR, Chess-Williams R. Which muscarinic receptor is important in the bladder? World Journal of Urology. 2001;**19**(5):299-306. DOI: 10.1007/s003450100226

[56] Jolliet P, Bourin M. Urosélectivité de l'antagonisme alpha-1 dans le traitement de l'hypertrophie bénigne de la prostate: du concept pharmacologique à l'approche clinique [Uroselectivity of alpha-1 antagonism in the treatment of benign prostatic hypertrophy: On the pharmacologic concept of the clinical approach]. Thérapie. 1998;**53**(1):61-66

[57] Williams LT, Mullikin D, Lefkowitz RJ. Identification of alpha-adrenergic receptors in uterine smooth muscle membranes by

[3H]dihydroergocryptine binding. Journal of Biological Chemistry. 1976;**251**(22):6915-6923

[58] Kitazawa T, Hirama R, Masunaga K, Nakamura T, Asakawa K, Cao J, et al. Muscarinic receptor subtypes involved in carbachol-induced contraction of mouse uterine smooth muscle. Naunyn-Schmiedeberg's Archives of Pharmacology. 2008;**377**(4-6):503-513. DOI: 10.1007/s00210-007-0223-1

[59] Bellavía S, Gallará R. Effect of photic stimuli on rat salivary glands. Role of sympathetic nervous system. Acta Odontológica Latinoamericana. 2000;**13**(1):3-19

[60] Proctor GB, Carpenter GH. Regulation of salivary gland function by autonomic nerves. Autonomic Neuroscience. 2007;**133**(1):3-18. DOI: 10.1016/j.autneu.2006.10.006

[61] Bone RC, Hiller C. Modern treatment of bronchial asthma. JACEP. 1978;**7**(7):269-275. DOI: 10.1016/s0361-1124(78)80337-2

[62] Goldie RG. Receptors in asthmatic airways. The American Review of Respiratory Disease. 1990;**141**(3 Pt 2):S151-S156. DOI: 10.1164/ajrccm/141.3_Pt_2.S151

[63] Goldie RG, Grayson PS, Knott PG, Self GJ, Henry PJ. Predominance of endothelinA (ETA) receptors in ovine airway smooth muscle and their mediation of ET-1-induced contraction. British Journal of Pharmacology. 1994;**112**(3):749-756. DOI: 10.1111/j.1476-5381.1994.tb13142.x

[64] Hu Y, Converse C, Lyons MC, Hsu WH. Neural control of sweat secretion: A review. The British Journal of Dermatology. 2018;**178**(6):1246-1256. DOI: 10.1111/bjd.15808

[65] Longmore J, Bradshaw CM, Szabadi E. Effects of locally and systemically administered cholinoceptor antagonists on the secretory response of human eccrine sweat glands to carbachol. British Journal of Clinical Pharmacology. 1985;**20**(1):1-7. DOI: 10.1111/j.1365-2125.1985.tb02791.x

[66] Shepherd JT. Circulatory response to beta-adrenergic blockade at rest and during exercise. The American Journal of Cardiology. 1985;**55**(10):87D-94D

[67] Mussina K, Toktarkhanova D, Filchakova O. Nicotinic acetylcholine receptors of PC12 cells. Cellular and Molecular Neurobiology. 2021;**41**(1):17-29. DOI: 10.1007/s10571-020-00846-x

[68] Exton JH. Mechanisms of hormonal regulation of hepatic glucose metabolism. Diabetes/Metabolism Reviews. 1987;**3**(1):163-183. DOI: 10.1002/dmr.5610030108

[69] LiverTox: Clinical and Research Information on Drug-Induced Liver Injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012. Beta-2 Adrenergic Agonists. 2017 Sep 11

[70] Yang LK, Tao YX. Physiology and pathophysiology of the β3-adrenergic receptor. Progress in Molecular Biology and Translational Science. 2019;**161**:91-112. DOI: 10.1016/bs.pmbts.2018.09.003

[71] Lynch GS, Ryall JG. Role of beta-adrenoceptor signaling in skeletal muscle: Implications for muscle wasting and disease. Physiological Reviews. 2008;**88**(2):729-767. DOI: 10.1152/physrev.00028.2007

[72] Soendenbroe C, Heisterberg MF, Schjerling P, Kjaer M, Andersen JL, Mackey AL. Human skeletal muscle acetylcholine receptor gene expression

in elderly males performing heavy resistance exercise. American Journal of Physiology. Cell Physiology. 2022;**323**(1):C159-C169. DOI: 10.1152/ajpcell.00365.2021

[73] Wen S, Li C, Zhan X. Muti-omics integration analysis revealed molecular network alterations in human nonfunctional pituitary neuroendocrine tumors in the framework of 3P medicine. The EPMA Journal. 2022;**13**(1):9-37. DOI: 10.1007/s13167-022-00274-5

[74] Golubnitschaja O, Potuznik P, Polivka J Jr, Pesta M, Kaverina O, Pieper CC, et al. Ischemic stroke of unclear aetiology: A case-by-case analysis and call for a multi-professional predictive, preventive and personalised approach. The EPMA Journal. 2022;**13**(4):535-545. DOI: 10.1007/s13167-022-00307-z

[75] Koklesova L, Mazurakova A, Samec M, Kudela E, Biringer K, Kubatka P, et al. Mitochondrial health quality control: Measurements and interpretation in the framework of predictive, preventive, and personalized medicine. The EPMA Journal. 2022;**13**(2):177-193. DOI: 10.1007/s13167-022-00281-6

[76] Evsevieva M, Sergeeva O, Mazurakova A, Koklesova L, Prokhorenko-KolomoytsevaI,ShchetininE, et al. Pre-pregnancy check-up of maternal vascular status and associated phenotype is crucial for the health of mother and offspring. The EPMA Journal. 2022;**13**(3):351-366. DOI: 10.1007/s13167-022-00294-1

[77] Kanaka V, Proikakis S, Drakakis P, Loutradis D, Tsangaris GT. Implementing a preimplantation proteomic approach to advance assisted reproduction technologies in the framework of predictive, preventive, and personalized medicine. The EPMA Journal. 2022;**13**(2):237-260. DOI: 10.1007/s13167-022-00282-5

[78] Xu J, Zhou H, Cheng Y, Xiang G. Identifying potential signatures for atherosclerosis in the context of predictive, preventive, and personalized medicine using integrative bioinformatics approaches and machine-learning strategies. The EPMA Journal. 2022;**13**(3):433-449. DOI: 10.1007/s13167-022-00289-y

[79] Kurysheva NI, Rodionova OY, Pomerantsev AL, Sharova GA, Golubnitschaja O. Machine learning-couched treatment algorithms tailored to individualized profile of patients with primary anterior chamber angle closure predisposed to the glaucomatous optic neuropathy. The EPMA Journal. 2023;**14**(3):527-538. DOI: 10.1007/s13167-023-00337-1

Chapter 2

Translational Genetics in Hereditary Cancers for Personalized Medicine Practice

Sadia Ajaz

Abstract

Elucidation of the molecular and cellular basis of disease has ushered in the era of personalized medicine. Current data indicate that 5–10% of cancers are hereditary, that is, caused by germline mutations in cancer predisposition genes. Examples include hereditary breast and ovarian cancers, von Hippel-Lindau syndrome, Cowden syndrome, and Lynch syndrome. The discoveries of causative genetic mutations have been possible due to the identification of families with a preponderance of cancers. After identifying patients with hereditary cancers through accurate family history, the next step is to identify the causative mutation(s) in these high-risk patients and individuals. Such detection can inform treatment; for instance, BRCA1/2 mutation carriers have now the option of intervention with Poly-ADP Ribose Polymerase (PARP) inhibitors such as olaparib and niraparib. It is important to emphasize that the study of hereditary forms of cancers has led to better treatment options not only for these cancers but also for the nonhereditary forms, which are more common. As next-generation sequencing becomes more affordable, it is likely that more causative mutations will be identified. This information can be translated for the development of either preventive or more suitable treatment strategies in cancers.

Keywords: inherited cancer syndrome, clinical application, precision medicine, germline mutation, early diagnosis, prevention, pharmacogenomics

1. Introduction

See **Box 1**.

Vignette: At a family gathering comprising members of four generations, the history of cancer in the family is brought up. During the conversation, the uterine cancer of the host (X)'s mother came up. It turns out that X's great paternal grandmother had died of unknown cancer, her paternal grandfather and uncle had died of throat cancer while a sister of her paternal grandfather had passed away due to colon cancer. One of X's maternal aunt had died of uterine cancer. Soon after the gathering, X is diagnosed with colon cancer at the age of 39 years. In a gene panel assay, a germline deletion mutation is found in X's *MLH1* gene.

Box 1.
Example of familial cancer history leading to the identification of causative germline mutation.

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The first observation that cancers could have a hereditary component was reported in 1866 [1], followed by clinical observations that hereditary cancers are characterized by features such as younger age and presentation in many members of the family [2, 3]. Additionally, strong hereditary cancer component is supported by epidemiologic evidence from twin studies demonstrating concordance for most cancers among monozygotic compared with dizygotic twins and siblings [4].

Cancer as a genetic disease has two heritable properties: (1) the cancer cells reproduce despite normal restraints of cell growth and division, and (2) these aberrant cells have the potential to metastasize, that is, invade and colonize tissues normally reserved for other cells [5].

The study of hereditary cancers has resulted in enormous progress in molecular oncology, such as the discovery of cancer genes, which play a major role in the hallmarks of cancer (**Figure 1**).

According to recent estimates, 5–10% of the cancers are hereditary, that is, caused by germline mutations in cancer predisposition genes, which are passed on from parent to offspring [8]. Two major categories of cancer-specific genes are tumor suppressor genes (TSGs) and oncogenes (**Figure 2**).

The study of familial cancer syndromes lead to the discovery of TSGs. In this category of genes, loss-of-function mutations result in abnormal cellular proliferation. The recessive alleles, present in a single copy in the germline, are not lethal, and the wild-type dominant allele needs to be inactive for the disease to manifest. Such a loss happens scarcely, thus the induction of phenotype, that is, cancer, is delayed until late in development.

In contrast to the TSGs, the oncogenes are activated by gain-of-function mutations. Thus, oncogenes function as dominant alleles in cells and are, therefore, usually lethal during normal embryonic development. Studies on hereditary cancers have

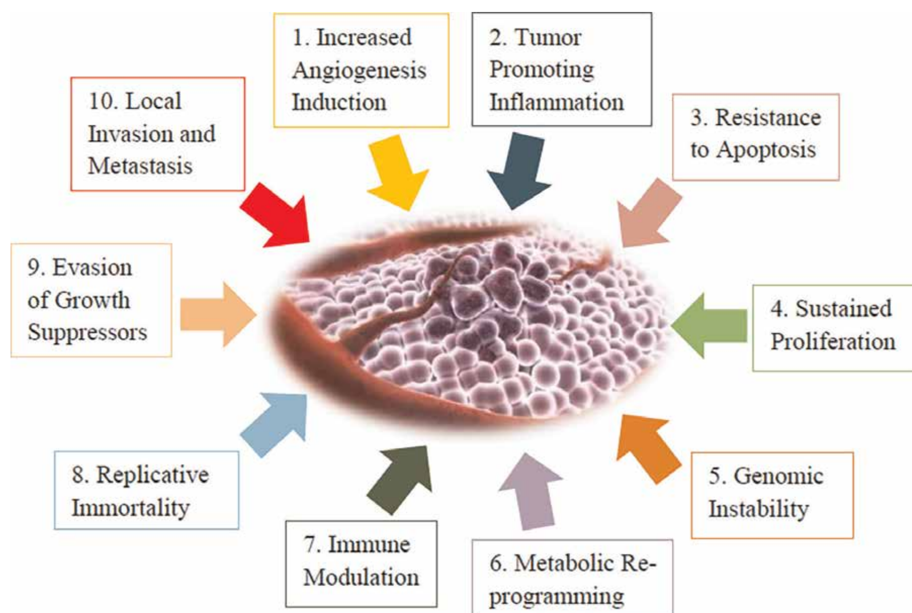

Figure 1.
Hallmarks of Cancers (adapted from: [6, 7]).

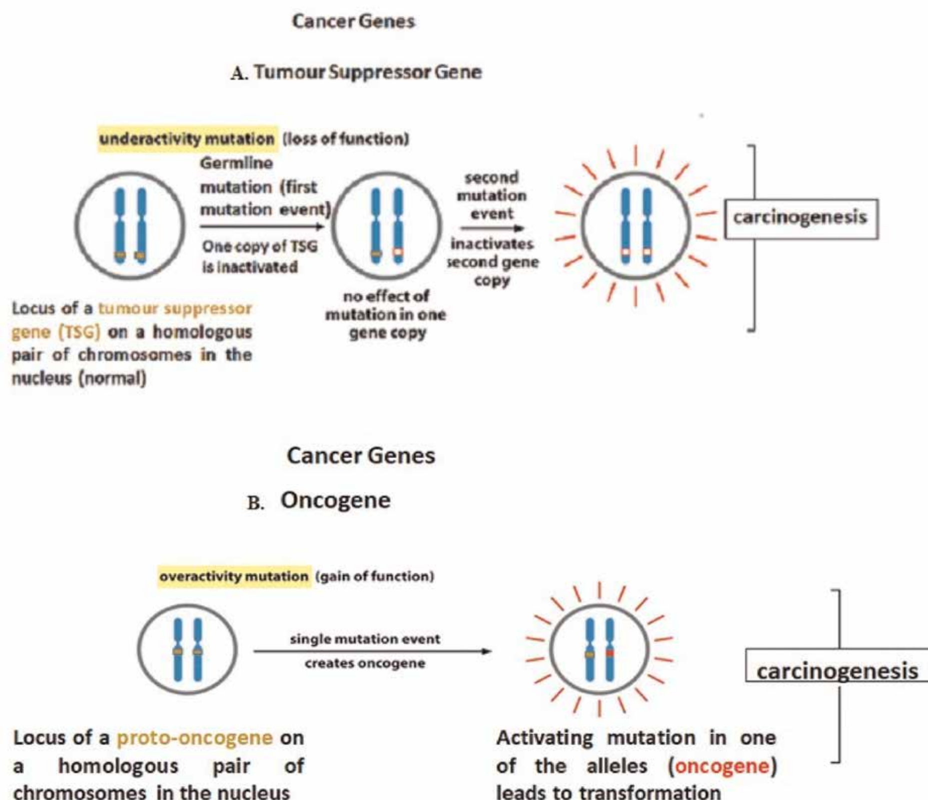

Figure 2.
Cancer genes. (A) Tumor suppressor genes. (B) Oncogenes (adapted from: [5]).

provided clues that constitutively active mutant oncogenes are generally not tolerated in the germline [9].

The potential of these insights needs to be recognized and applied not only in disease treatment but even more importantly in disease prevention.

Regrettably, most of the anti-cancer treatments being deployed today were developed prior to 1975, especially in developing countries. The investigation of inherited cancers has contributed to the development of a multitude of better anti-cancer treatments. Moreover, with advancements in sequencing technologies, the number of cancers with known underlying genetic mutations is likely to increase [10]. These discoveries are likely to result in translational applications. The defective molecular mechanisms serve as therapeutic targets, for example, mutant alleles of certain tyrosine kinase receptor genes. Such mutations are rare and can be transmitted in the human germline. Targets like these and others have enabled pharmaceutical companies and biotechnology firms to develop therapeutic strategies such as low-molecular-weight drugs, proteins, monoclonal antibodies, gene therapies, and more recently, gene editing.

These extraordinary leaps in the understanding of cancer biology have revolutionized the field of oncology, referred to as precision oncology. Some of the inherited cancer syndromes, their associated genes, along with their translational applications, are listed in **Table 1**.

Sr. no.	Inherited cancer syndrome	Primary tumor	Inheritance/associated cancers	Chromosomal location	Underlying gene(s)	Role in carcinogenesis	Translational applications
1.	Hereditary breast and ovarian cancers (HBOCs)	Breast and ovarian cancers	Prostate and pancreatic cancers	17q21	*BRCA1*	DNA repair	PARP inhibitors [5].
				13q12.3	*BRCA2*		
2.	Hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome	Colorectal cancer	Autosomal Dominant inheritance/endometrial, ovarian, hepatobiliary and urinary tract cancer glioblastoma (Turcot syndrome)	2p21-p16.3	*MSH2*	Tumor Suppressors	*Defective mismatch repair*. Precision medicine targeting immune-based mechanisms is likely to be promising (anti–PD-1 monoclonal antibodies (pembrolizumab or nivolumab) yield 70% or greater disease control rates [11].
				2p16.3	*MSH6*		
				7p22.1	*PMS2*		
3.	Familial adenomatous polyposis (FAP)	Colorectal cancer	Autosomal dominant/recessive. Colorectal adenomas, duodenal and gastric tumors, congenital hypertrophy of the retinal pigment epithelium (CHRPE), jaw osteomas and desmoid tumors (Gardner syndrome) medulloblastoma (Turcot syndrome)	5q22.2	*APC*	Tumor suppressor	1. Surveillance from the age of 10–12 years 2. Annual flexible sigmoidoscopy or colonoscopy until polyps are detected. Then, annual colonoscopy until colectomy is done [12]. 3. Anti-inflammatory drug sulindac can serve as a chemoprevention therapy 4. Combinations with sulindac can be tried, including the targeted therapy erlotinib or difluoromethylornithine (DFMO in short) [13].
4.	von Hippel–Lindau (VHL) syndrome	Renal cancer (clear cell)	Autosomal dominant inheritance/ pheochromocytomas, retinal angiomas, and hemangioblastomas	3p25.3	*VHL*	Tumor suppressor	1. Renal cell carcinoma/ pancreatic neuroendocrine tumors: *surveillance from age 10 years – biannual* abdominal MRI with abdominal ultrasound in the intervening years.

Sr. no.	Inherited cancer syndrome	Primary tumor	Inheritance/associated cancers	Chromosomal location	Underlying gene(s)	Role in carcinogenesis	Translational applications
							2. Pheochromocytoma – surveillance from age 2 years a. Annual physical examination with blood pressure monitoring. b. Annual fasting-free plasma metanephrines and if available plasma 3-methoxytyramine (if plasma unavailable 24-hour urine-free metanephrines) [14] 3. Belzutifan (HIF2α inhibitor) acts on a downstream target of *VHL* [15].
5.	Hereditary papillary renal cancer (HPRC)	Renal cancer (papillary type)	Susceptibility to osteofibrous dysplasia	7q31.2	*MET*	*Oncogene*	1. Telisotuzumab vedotin, an anti-MET antibody conjugated with monomethyl auristatin E (a tubulin polymerization inhibitor), has shown favorable antitumor activity in patients with NSCLC and MET overexpression and is being investigated in a phase II trial (NCT03539536) 2. *MET* kinase inhibitors (e.g., bozitinb, cabozantinib, and crizotinib are in clinical trials) [16]

Sr. no.	Inherited cancer syndrome	Primary tumor	Inheritance/associated cancers	Chromosomal location	Underlying gene(s)	Role in carcinogenesis	Translational applications
6.	Familial melanoma	Melanoma	Autosomal dominant inheritance/pancreatic cancer, dysplastic nevi, atypical moles	9p21	*CDK2NA* (p16)	Tumor suppressor	1. Surveillance from age 18 years Recommendations include: • Monthly self-examination of skin • 6 monthly comprehensive skin examinations, including scalp and genitalia, by a dermatologist, supplemented by total body photography and dermoscopy • Minimize sun exposure and follow sun-smart practices [17] 2. Studies indicate that CDK2NA mutation is associated with poor response to anti-PD-1/PD-L1 antibodies (a class of immune checkpoint inhibitors) [18].
7.	Fanconi's anemia (A-W) [19]	Acute myeloid leukemia (AML)	Autosomal/X-linked recessive inheritance. Pancytopenia, skeletal abnormalities, diverse carcinomas.	17q23.2	A total of 13 genes have been cloned, and at least 13 complementation groups have been demonstrated. 1. Complementation group J encodes the BACH1 (BRIP1) protein.	DNA repair	The replacement of an abnormal gene by a normal gene is the modern developing technique. The correction of CD34+ in affected cells is now feasible [20]. For *FANCN* (*PALB2*): *Breast Cancer*: • Females: annual mammograms alternating with MRI for individuals (each once per year) as well as clinical breast exam twice yearly beginning at 30 years of age for women with a
				13q13.1	2. A number of products of the FANC genes form a complex that interacts with BRCA1 and its partners BRCA2 associates with		

Sr. no.	Inherited cancer syndrome	Primary tumor	Inheritance/associated cancers	Chromosomal location	Underlying gene(s)	Role in carcinogenesis	Translational applications
					BRCA1 (and FANCD1 = BRCA2). Homozygous absence of either the RAD51C, FANCD1 (= BRCA2), FANCJ (= BACH1), or FANCN (= PALB2) gene leads to Fanconi anemia, while lack of only one gene copy leads to breast cancer and/or susceptibility thereto. [23, 24]		*PALB2* variant. Consider risk-reducing mastectomy based on family history. Consider medication to reduce the risk of developing breast cancer for women not planning bilateral mastectomy within 3 years: ◦ Pre-menopausal women may consider tamoxifen ◦ Post-menopausal women may consider raloxifene, aromatase inhibitors, or tamoxifen • Males: Self-exam training and education with a clinical breast exam every 12 months starting at age 35 + in men with pathogenic *PALB2* variant. Consider annual mammogram screening in men with gynecomastia starting at age 50 or 10 years before the earliest known male breast cancer in the family. *Ovarian:* • Consider risk-reducing salpingo-oophorectomy (RRSO) at age > 45yo. *Pancreatic cancer*: • Screen P/LP variant carriers with a family history of cancer [21, 22].
				16q24.3 (Autosomal recessive)	*FANCA*		
				Xp22.2 (X-Linked Recessive)	*FANCB*		
				9q22.32	*FANCC*		
				3p25.3	*FANCD2*		
				6p21.31	*FANCE*		
				11p14.3	*FANCF*		
				9p13.3	*FANCG*		
				15q26.1	*FANCI*		
				2p16.1	*FANCL*		
				14q21.2	*FANCM*		
				16p12.2	*FANCN*		

Sr. no.	Inherited cancer syndrome	Primary tumor	Inheritance/associated cancers	Chromosomal location	Underlying gene(s)	Role in carcinogenesis	Translational applications
8.	Ataxia telangiectasia (AT)	Leukemia/ lymphoma	Autosomal recessive inheritance, Cerebellar ataxia, immunodeficiency, breast cancer in heterozygotes.	11q22.3	*ATM*	DNA repair	Mutation-specific recommendations [25]. • Several ATM inhibitors are in Phase I clinical trials, e.g., KU60019 [26] and KU55933 [27].
9.	Bloom syndrome	Leukemias/ lymphomas,	Autosomal recessive inheritance, multiple solid tumors	15q26.1	*RECQL3* (*BLM*)	DNA repair	*Specific surveillance strategies at earlier ages [28].*
10.	Xeroderma pigmentosum (XP) groups A–G	Skin cancer	Autosomal recessive	9q22.33	*XPA*	DNA repair	Recently, oral vismodegib (hedgehog pathway inhibitor), pembrolizumab (PD-1 inhibitor), nivolumab (PD-1 inhibitor) and cemiplimab (PD-1 inhibitor) have been used for the treatment of melanoma and non-melanoma skin cancers, and the preliminary results are encouraging [29]. Multiple melatonin agonists [30] are available, and N-acetyl-5-methoxytryptamine (NPC15) is effective in suppressing skin tumor development. It is now in clinical trials [31].
				2q14.3	*XPB* (*ERCC3*)		
				3p25.1	*XPC*		
				19q13.32	*XPD*		
				11q12.2	*XPE-BF* (*DDB1*)		
				16p13.12	*XPF*		
				13q33.1	*XPG* (*ERCC5*)		
				6p21.1	*XP-V* (*POLH*)		
11.	Nijmegen breakage	Mostly lymphomas	Autosomal recessive inheritance/similar to AT (cerebellar ataxia, immunodeficiency) breast cancer in heterozygotes (similar to ataxia)	8q21.3	*NBN*	DNA repair	Treatment of malignancy in NBS is particularly difficult since the underlying gene, nibrin (*NBN*), is involved in the cellular response to DNA damage.

Sr. no.	Inherited cancer syndrome	Primary tumor	Inheritance/associated cancers	Chromosomal location	Underlying gene(s)	Role in carcinogenesis	Translational applications
							The consequent hypersensitivity of NBS patients severely limits therapeutic options, and standard chemotherapeutics such as cyclophosphamide must be used at lower doses and radiotherapy is completely avoided [32].
12.	Wilms tumor	Wilms tumor	Autosomal dominant inheritance/WAGR (Wilms, aniridia, genitourinary abnormalities, mental retardation)	11p13	*WT1* Other predisposition genes have been identified [33]	Tumor suppressor	Molecular testing of tumor tissue (e.g., LOH and 1q gain) is recommended to use in risk assessment for all newly diagnosed patients with FHWT. The goal is to select therapy that will increase survival and decrease relapse, morbidity, and long-term adverse events. Patients are categorized as (1) very low-risk, (2) low-risk, (3) standard risk, (4) higher risk, and (5) bilateral [34].

Table 1.
Representative inherited cancer syndromes, the underlying genes, and the relevant translational applications.

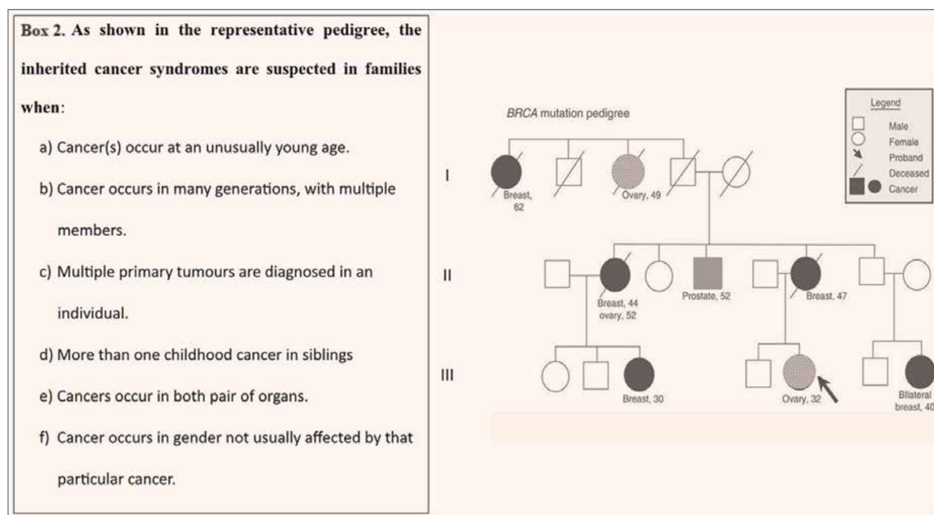

Figure 3.
Representative pedigree for inherited cancer syndromes (number represent age at diagnosis).

2. Inherited cancer syndromes

The salient features of inherited cancer syndromes in general along with a representative pedigree are presented in **Figure 3**.

The characteristic hereditary cancer syndromes with overarching translational applications in the corresponding sporadic cases are discussed in this chapter.

3. Hereditary breast and ovarian cancers (HBOCs)

According to the data collected in 2020, breast cancers were diagnosed in 2.3 million women, while it accounted for 685,000 deaths globally. Data from 2015 to 2020 show that 7.8 million women with breast cancer were alive during this period, making it the world's most prevalent cancer [35]. According to Globocan 2018, the incidence of ovarian cancer was 295,414 new cases, with a total of 184,799 deaths [36]. According to conventional estimates, 5–10% of breast cancer cases have genetic predisposition [37]. With advancements in technology, the breast cancer heritability due to known genetic risk loci may approach up to 30% [38].

The general features of HBOCs and a sample pedigree are presented in **Figure 3**. Specifically, a diagnosis of HBOC is suspected in young women and when members of the same side of the family present with multiple cases of breast cancer and/or ovarian cancers. The chance that a family has HBOC increases in any of the situations [39, 40] listed below:

- One or more women are diagnosed at age ≤ 45 years.
- Triple-negative breast cancer in women at age ≤ 60 years.
- A woman is diagnosed with bilateral breast cancer or multiple primary breast cancers, or she is diagnosed with both breast and ovarian cancers.

- One or more women are diagnosed with breast cancer <50 years of age with an additional family history of cancer (e.g., melanoma, prostate, and pancreatic cancer).
- There are breast and/or ovarian cancers in multiple generations on the same side of the family, such as having both a paternal grandmother and an aunt on the father's side diagnosed with these cancers.
- In addition to a history of breast and ovarian cancer, prostate and/or pancreatic cancer are diagnosed on the same side of the family.
- Breast cancer in a male relative.
- Having Ashkenazi Jewish ancestry

3.1 *BRCA1* and *BRCA2*

Approximately 30 years back, germline mutations in two genes (*BRCA1* and *BRCA*2) were linked with hereditary cases of breast and ovarian cancers. The loci of the *BRCA1* and *BRCA2* genes were mapped to chromosomes 17q21 and 13q12.3, respectively [41, 42]. Both sites often exhibited loss of heterozygosity (LOH) in familial and sporadic tumors. In familial cases, it was observed that consequent to LOH at these loci, there was the sole retention of the disease-predisposing mutant allele [43, 44].

With accumulating evidence over the decades, it is recommended that testing for actionable mutations in *BRCA1* and *BRCA2* should be offered to healthy women with a severe family history of breast or ovarian cancer. The cancer risk for mutation carriers can be significantly reduced by surgical intervention [45, 46].

Even in the absence of a familial history, carriers of *BRCA1* and *BRCA2* mutations in the general population have an increased risk of breast or ovarian cancer by the age of 80 years: 83% for *BRCA1* mutation carriers and 76% for *BRCA2* mutation carriers [47].

3.2 Translational applications of *BRCA1/2* screening (PARP inhibitors)

As shown in **Figure 1**, cancer cells require genetic instability to evolve and proliferate. However, the same property can be targeted to kill these cells. Normal cells require DNA repair mechanisms, which allow them to perform their functions despite DNA damage (even under normal circumstances, cells accumulate mutations at the rate of three per gene per cell division). One of the DNA maintenance mechanisms is the repair of double-stranded chromosomal breaks by homologous recombination, in which *BRCA1* and *BRCA2* are involved. Single-strand breaks instead are repaired by a mechanism in which polyADP-ribose polymerase (PARP) is a critical enzyme. This basic mechanism led to a striking discovery: drugs that block PARP activity kill *BRCA*-deficient cells (i.e., cells in which both copies of either *BRCA1* or their *BRCA2* tumor suppressor genes are inactivated) with extraordinary selectivity. At the same time, PARP inhibition has very little effect on normal cells [5].

In December 2016, an accelerated approval for a PARP inhibitor, rucaparib (Rubraca™) to treat advanced ovarian cancer was granted by the Food and Drug Administration (FDA).

The drug is approved for women whose tumors have a mutation in the *BRCA1* or *BRCA2* genes, as identified by an FDA-approved companion diagnostic test, and their cancers have progressed despite treatment with two or more chemotherapies. About 15–20% of women with ovarian cancer harbor a *BRCA* mutation [48].

3.3 Breast cancer in Li-Fraumeni syndrome

Li-Fraumeni syndrome (LFS) is rare, with an autosomal-dominant mode of inheritance. It is mainly associated with germline mutations in the tumor suppressor gene, *TP53* [49]. It is one of the most frequently mutated genes in cancers. *TP53* encodes the protein p53, which also has an important role in the regulation of the cell cycle. The risk of early onset malignancy is increased in the carriers of *TP53* germline mutations [50]. Carcinoma of the breast is the most common malignancy in women who inherit *TP53* mutations [51]. Mai et al. report an early onset of breast cancer around the age of 40 years among women with *TP53* mutation [52]. Another study, which was published earlier, reported the median age of breast cancer diagnosis at 34 years [53]. It is estimated that germline *TP53* mutations are present in the range of approximately 5–8% of women <30 years of age who are diagnosed with breast cancer [54]. By the age of 60 years, female *TP53* mutation carriers have an 85% cumulative lifetime risk of breast cancer, which is comparable to that of *BRCA1* and *BRCA2* mutation carriers [52].

Long-term survival may be improved in the carriers of pathogenic *TP53* variants by a comprehensive surveillance protocol for early tumor detection as indicated by cumulative evidence. This surveillance protocol was based on multiple modalities such as physical examination, blood tests, and different imaging platforms. According to the guidelines, provided the logistics, this approach should be considered in the clinical management of these patients [55].

3.3.1 Translational applications of TP53 sequencing in breast cancers associated with Li-Fraumeni syndrome

In cases of germline *TP53* actionable mutation, instead of breast conserving therapy (BCT), mastectomy is recommended. This surgical intervention reduces the risks of a second primary breast cancer. Where feasible, radiotherapy needs to be avoided. Depending on the patient's age, bilateral mastectomy should also be considered as the risk of contralateral breast cancer is increased [54].

Among *TP53* mutation carriers, there are concerns about an increased risk of radiation-induced second primary tumors. Therefore, in the UK, mammography is not recommended, and annual MRI breast screening is recommended from age 20 to 49 years, while guidelines suggest that between 50 and 69 years, annual MRI should be considered [56]. In the USA, National Comprehensive Cancer Network (NCCN) guidelines suggest annual breast MRI between 20 and 29 years. The older carriers are recommended for annual MRI and mammography [57]. In Australia, according to national guidelines, bilateral mastectomy should be offered, and in case the carriers prefer other options, annual breast MRI is recommended from 20 years onward [58].

3.4 Other genes implicated in breast cancer risk

A comprehensive list of genes, where the variants increase the breast cancer predisposition, is provided in **Table 2**.

High penetrance (high-risk genes)			
Sr. no.	**Gene**	**Gene details**	**Lifetime risk of breast cancer**
1.	*BRCA1*	*BREAST CANCER 1*: Maintenance of genomic stability (genomic instability and mutation).	65% by age 70 years
2.	*BRCA2*	*BREAST CANCER 2*: Both *BRCA1* and *BRCA2* are involved in the maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair (genomic instability and mutation).	45% by age 70 years
3.	*TP53*	*TUMOR PROTEIN P53*: tumor suppressor (evading growth suppressors).	85% by age 60 years
4.	*PTEN*	*Phosphatase and Tensin Homolog:* This gene is identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded by this gene is phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. It contains a tensin-like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, this protein preferentially dephosphorylates phosphor-inositide substrates (evading growth suppressors).	67–87%
5.	*STK11*	*Serine/Threonine Kinase 11 (Polarization-Related Protein LKB1) (Renal Carcinoma Antigen NY-REN-19) (Liver Kinase B1):* This gene, which encodes a member of the serine/threonine kinase family, regulates cell polarity and functions as a tumor suppressor. Mutations in this gene have been associated with Peutz-Jeghers syndrome, an autosomal dominant disorder characterized by the growth of polyps in the gastrointestinal tract, pigmented macules on the skin and mouth, and other neoplasms. Alternate transcriptional splice variants of this gene have been observed but have not been thoroughly characterized (evading growth suppressors).	44–50% by age 70 years
6.	*CDH1*	*Cadherin 1 (E-Cadherin)*: This gene encodes a classical cadherin of the cadherin superfamily. Alternative splicing results in multiple transcript variants, at least one of which encodes a pre-proprotein that is proteolytically processed to generate the mature glycoprotein. This calcium-dependent cell-cell adhesion protein comprises five extracellular cadherin repeats, a transmembrane region, and a highly conserved cytoplasmic tail (activating invasion and metastasis).	42–60%
7.	PALB2	*Partner and Localizer of BRCA2 (Fanconi Anemia, Complementation Group N) (FANCN) (PNCA3):* This gene encodes a protein that may function in tumor suppression. This protein binds to and co-localizes with the breast cancer 2 early onset protein (*BRCA2*) in nuclear foci and likely permits the stable intra-nuclear localization and accumulation of *BRCA2* (evading growth suppressors).	33% by age 70 without a family history or 58% by age 70 with a family history

High penetrance (high-risk genes)			
Sr. no.	**Gene**	**Gene details**	**Lifetime risk of breast cancer**
Moderate penetrance (moderate risk genes)			
1.	*ATM*	*Ataxia Telangiectasia Mutated Serine/Threonine Kinase*: This protein is an important cell cycle checkpoint kinase that phosphorylates (enabling replicative immortality).	20%
2.	*CHEK2*	*Checkpoint Kinase 2 (RAD53)*: In response to DNA damage and replication blocks, cell cycle progression is halted through the control of critical cell cycle regulators. The protein encoded by this gene is a cell cycle checkpoint regulator and putative tumor suppressor (enabling replicative immortality and resisting cell death).	20–25%
3.	*BARD1*	*BRCA1 Associated RING Domain 1*: In addition to its ability to bind BRCA1 *in vivo* and *in vitro*, it shares homology with the two most conserved regions of BRCA1: the N-terminal RING motif and the C-terminal BRCT domain (evading growth suppressors and genomic instability and mutation).	21% (Caucasians) – 39% (African-American)* *Triple negative breast cancer
4.	*RAD51D*	*RAD51 Paralog D (RAD51-Like Protein 3), DNA Repair Protein RAD51 Homolog 4*: The protein encoded by this gene is a member of the RAD51 protein family. *RAD51* family members are highly similar to bacterial RecA and *Saccharomyces cerevisiae* Rad51, which are known to be involved in the homologous recombination and repair of DNA. This protein forms a complex with several other members of the RAD51 family, including *RAD51L1*, *RAD51L2*, and *XRCC2*. The protein complex formed with this protein has been shown to catalyze homologous pairing between single- and double-stranded DNA and is thought to play a role in the early stage of recombinational repair of DNA. Alternative splicing results in multiple transcript variants. Read-through transcription also exists between this gene and the downstream ring finger and FYVE-like domain containing 1 (*RFFL*) gene (genomic instability and mutation).	19% (without family history) 44% (with family history)
5.	*RAD51C*	*RAD51 Paralog C (RAD51-Like Protein 2), (REC2), DNA Repair Protein RAD51 Homolog 3*: This gene is a member of the RAD51 family. RAD51 family members are highly similar to bacterial RecA and *Saccharomyces cerevisiae* Rad51 and are known to be involved in the homologous recombination and repair of DNA. This protein can interact with other *RAD51* paralogs and is reported to be important for Holliday junction resolution. Mutations in this gene are associated with Fanconi anemia-like syndrome. This gene is one of the four localized to a region of chromosome 17q23 where amplification occurs frequently in breast tumors. Overexpression of the four genes during amplification has been observed and suggests a possible role in tumor progression. Alternative splicing results in multiple transcript variants (genomic instability and mutation).	20% (without family history) 46% (with family history)

High penetrance (high-risk genes)			
Sr. no.	**Gene**	**Gene details**	**Lifetime risk of breast cancer**
6.	*NBN*	*Nijmegen Breakage Syndrome 1 (Nibrin):* Mutations in this gene are associated with the encoded protein which is a member of the MRE11/RAD50 double-strand break repair complex which consists of five proteins. This gene product is thought to be involved in DNA double-strand break repair and DNA damage-induced checkpoint activation (genomic instability and mutation).	23%
Low penetrance (low-risk genes)			
1.	*ATR*	*Ataxia Telangiectasia And Rad3-Related Protein Serine/Threonine Kinase (FRAP-Related Protein-1)*: The protein encoded by this gene is a serine/threonine kinase and DNA damage sensor, activating cell cycle checkpoint signaling upon DNA stress (resisting cell death and genomic instability and mutation).	More data needed
2.	*BAP1*	*BRCA1 Associated Protein 1*: Ubiquitin C-terminal hydrolase subfamily of deubiquitinating enzymes that are involved in the removal of ubiquitin from proteins. The encoded enzyme binds to the breast cancer type 1 susceptibility protein (BRCA1) via the ring finger domain of the latter and acts as a tumor suppressor (evading growth suppressors and genomic instability and mutation).	Insufficient data
3.	*BRIP1*	*BRCA1 Interacting Protein C-Terminal Helicase 1*: The protein encoded by this gene is a member of the RecQ DEAH helicase family and interacts with the BRCT repeats of breast cancer, type 1 (BRCA1). The bound complex is important in the normal double-strand break repair function of breast cancer, type 1 (BRCA1). This gene maybe a target of germline cancer-inducing mutations (genomic instability and mutation).	<20%
4.	*CHEK1*	*Checkpoint Kinase 1*: The protein encoded by this gene belongs to the Ser/Thr protein kinase family. It is required for checkpoint-mediated cell cycle arrest in response to DNA damage or the presence of unreplicated DNA. This protein acts to integrate signals from ATM and ATR, two cell cycle proteins involved in DNA damage responses, that also associate with chromatin in meiotic prophase I (enabling replicative immortality and resisting cell death).	More data needed
5.	*CTNNA1*	*Catenin (Cadherin-Associated Protein) Alpha 1*: This gene encodes a member of the catenin family of proteins that play an important role in the cell adhesion process by connecting cadherins located on the plasma membrane to the actin filaments inside the cell (activating invasion and metastasis).	More data needed
6.	*FAM175A*	*Family with Sequence Similarity 175 Member A (Abraxas 1, BRCA1 A Complex Subunit)*: This gene encodes a protein that binds to the C-terminal repeats of breast cancer 1 (*BRCA1*) through a phospho-SXXF motif. The encoded protein recruits ubiquitin interaction motif	More data needed

High penetrance (high-risk genes)			
Sr. no.	**Gene**	**Gene details**	**Lifetime risk of breast cancer**
		containing 1 protein to *BRCA1* protein and is required for DNA damage resistance, DNA repair, and cell cycle checkpoint control. (genomic instability and mutation).	
7.	*FANCM*	*Fanconi Anemia Complementation Group M (ATP-Dependent RNA Helicase FANCM)*: The Fanconi anemia complementation group (FANC) currently includes FANCA, FANCB, FANCC, FANCD1 (also called *BRCA2*), FANCD2, FANCE, FANCF, FANCG, FANCI, FANCJ (also called BRIP1), FANCL, FANCM and FANCN (also called PALB2). The previously defined group FANCH is the same as FANCA. Fanconi anemia is a genetically heterogeneous recessive disorder characterized by cytogenetic instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair (genomic instability and mutation).	More data needed
8.	*GEN1*	*GEN1, Holliday Junction 5' Flap Endonuclease*: This gene encodes a member of the Rad2/xeroderma pigmentosum group G nuclease family, whose members are characterized by N-terminal and internal xeroderma pigmentosum group G nuclease domains followed by helix-hairpin-helix domains and disordered C-terminal domains. The protein encoded by this gene is involved in the resolution of Holliday junctions, which are intermediate four-way structures that covalently link DNA during homologous recombination and double-strand break repair.	More data needed
9.	*MRE11A*	*Meiotic Recombination 11 Homolog A Double Strand Break Repair Nuclease:* This gene encodes a nuclear protein involved in homologous recombination, telomere length maintenance, and DNA double-strand break repair. By itself, the protein has 3′–5′ exonuclease activity and endonuclease activity (genomic instability and mutation).	More data needed
10.	*RAD51B*	*RAD51 Paralog B (RAD51 Homolog B) (REC2), DNA Repair Protein RAD51 Homolog 2*: The protein encoded by this gene is a member of the RAD51 protein family. RAD51 family members are evolutionarily conserved proteins essential for DNA repair by homologous recombination. This protein has been shown to form a stable heterodimer with the family member RAD51C, which further interacts with the other family members, such as RAD51, XRCC2, and XRCC3. Overexpression of this gene was found to cause cell cycle G1 delay and cell apoptosis, which suggested a role of this protein in sensing DNA damage. Rearrangements between this locus and high mobility group AT-hook 2 (HMGA2, GeneID 8091) have been observed in uterine leiomyomata (genomic instability and mutation).	More data needed
11.	*RECQL*	*RecQ Like Helicase [RecQ Protein-Like (DNA Helicase Q1-Like)]:* The protein encoded by this gene is a member of the RecQ DNA helicase family. DNA	More data needed

High penetrance (high-risk genes)			
Sr. no.	**Gene**	**Gene details**	**Lifetime risk of breast cancer**
		helicases are enzymes involved in various types of DNA repair, including mismatch repair, nucleotide excision repair, and direct repair. The encoded protein is involved in the processing of Holliday junctions, the suppression of sister chromatid exchanges, and telomere maintenance, and is required for genotoxic stress resistance. Defects in this gene have been associated with several types of cancer (genomic instability and mutation).	
12.	*RINT1*	*RAD50 Interactor 1:* This gene encodes a protein first identified for its ability to interact with the RAD50 double-strand break repair protein, with the resulting interaction implicated in the regulation of cell cycle progression and telomere length. The encoded protein may also play a role in the trafficking of cellular cargo from the endosome to the trans-Golgi network. Mutations in this gene maybe associated with breast cancer in human patients (evading growth suppressors, resisting cell death and genomic instability and mutation).	More data needed
13.	*SLX4*	*SLX4 Structure-Specific Endonuclease Subunit, (Fanconi Anemia, Complementation Group P, FANCP):* This gene encodes a protein that functions as an assembly component of multiple structure-specific endonucleases. These endonuclease complexes are required for the repair of specific types of DNA lesions and are critical for cellular responses to replication fork failure. Mutations in this gene were found in patients with Fanconi anemia (genomic instability and mutation).	More data needed
14.	*XRCC2*	*X-Ray Repair Cross Complementing 2, (X-Ray Repair Complementing Defective Repair In Chinese Hamster Cells 2), DNA Repair Protein XRCC2, RAD51-Like, FANCU (Fanconi Anemia Complementation Group U):* This gene encodes a member of the RecA/Rad51-related protein family that participates in homologous recombination to maintain chromosome stability and repair DNA damage. This gene is involved in the repair of DNA double-strand breaks by homologous recombination and it functionally complements Chinese hamster irs1, a repair-deficient mutant that exhibits hypersensitivity to a number of different DNA-damaging agents (evading growth suppressors).	More data needed

**Data compiled from established resources [57, 59–66].*

Table 2.
Genes implicated in increased susceptibility to breast cancers along with the function of encoded proteins.

These genes are categorized as high, moderate, or low-risk genes. Although, this classification is flexible as more data are generated. The high penetrance (or high risk) genetic variants increase the relative risk for cancer development >4 times as

compared to the general population. Translationally, the guidelines have been formulated for pathogenic variants of these genes by consortia [57, 59–66].

Pathogenic variants in moderate penetrance (or moderate risk) genes confer a 2–4 times relative risk. Low penetrance/risk genes are those with <2 times increased risk of cancer or those that have limited data indicating association and magnitude of cancer risk [8].

4. Familial colon cancers

Colorectal cancer accounts for approximately 10% of all cancer cases. In its frequency, it is the third most common cancer worldwide. It is the second leading cause of cancer-related deaths, globally. The incidence and mortality rates show large geographical variations. According to a World Health Organization estimate, there were > 1.9 million new cases and > 930,000 deaths due to colorectal cancer in 2020, globally [19]. Studies, including twin and kindred study designs, have indicated that more than 25–30% of colorectal cancer cases are familial. Among such cases, about 5% of all CRC cases are known to be associated with defined highly penetrant cancer syndromes at present [67].

The bulk of these cases are attributable to hereditary nonpolyposis colorectal cancer syndromes (HNPCCs) also known as Lynch syndrome, with another significant subset associated with the familial adenomatous polyposis (FAP) syndrome [68].

4.1 Familial adenomatous polyposis (FAP)

The major form of FAP syndrome is autosomal dominant, which affects about 1 in 8300 individuals in the United States [13] and accounts for less than 1% of CRCs [69]. The underlying genetic mutation is in the *adenomatous polyposis coli* (*APC*) tumor suppressor gene on chromosome 5q21 (**Table 1**) [70]. Among the studied FAP families, germline mutations have been identified in one *APC* allele of affected individuals in 90–95% of pedigrees [71, 72]. The consequent aberration in downstream pathways leads to carcinogenesis.

A less frequent, milder form of FAP has an autosomal recessive inheritance pattern, which is associated with the *MUTHY* gene. Thus, it is also known as MUTHY-associated polyposis (MAP). The gene on chromosomal locus 1p34.1 encodes a DNA glycosylase. The DNA damage repair enzyme removes adenine bases from the DNA backbone where it is inappropriately paired with guanine, cytosine, or 8-oxo-7,8-dihydroguanine, a major oxidatively damaged DNA lesion [73].

4.1.1 Translational implications

As shown in **Figure 4**, hundreds to thousands of adenomas arise in the large bowel and rectum beginning in the second decade of life in FAP. A small number of patients with adenomas progress to cancer development. However, in untreated FAP patients, the incidence of colorectal cancer over lifetime approaches 100%, with the mean age at diagnosis is 39 years. Thus, it becomes essential to remove the patient's colon early in life as a prophylactic measure. Additionally, therapeutic regimens should take into consideration that these cancers are sensitive to 5-flurouracil and resistant to anti-epidermal growth factor therapy.

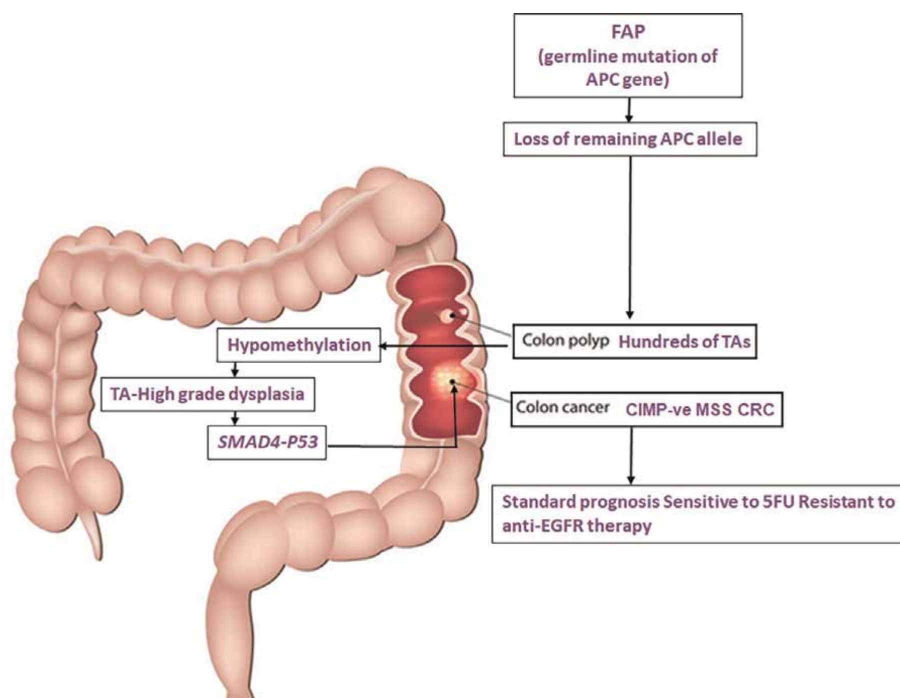

Figure 4.
Familial Adenomatous Polyposis (FAP) pathway to colorectal cancer (CRC). It shows the relationship of the FAP pathway lesions to CRC prognosis and therapeutic response. TA: tubular adenoma; CIMP–ve: CpG island methylator phenotype-negative; MSS: microsatellite stable; anti-EGFR: epidermal growth factor receptor inhibitors; 5-FU: 5-fluorouracil therapy.

4.2 Hereditary nonpolyposis colorectal cancer syndromes (HNPCCs)/lynch syndrome

Hereditary nonpolyposis colorectal cancer (HNPCC), now referred to as Lynch syndrome as well, is among the first inherited cancer syndromes to be well described in the literature. In 1913, Warthin reported a three-generation family with CRC and other cancers, including gastric cancer and tumors of the female reproductive tract [74]. Subsequently, Lynch and others described kindreds with autosomal dominant patterns of CRC, which were not characterized by extensive polyposis [75]. In such families, CRCs of early onset were seen, often along with cancers in some other organs including gastric, uterine endometrial, ovarian, small bowel, renal, and hepatobiliary cancers [74]. The germline mutations underlying HNPCC are involved in the mismatch repair (MMR) pathway, including *MSH2*, *MLH1,* and *PMS2* genes [76]. Accumulation of further mutations and dysregulation of subsequent pathways leads to CRCs.

4.2.1 Translational implications

These cancers have good prognosis. These are sensitive to anti-EGFR therapy but resistant to 5-Flurouracil (**Figure 5**). Checkpoint inhibitors targeting immune-based mechanisms are likely to be promising. Pembrolizumab and nivolumab (anti–PD-1 monoclonal antibodies) have 70% or greater disease control rates (**Table 1**) [11].

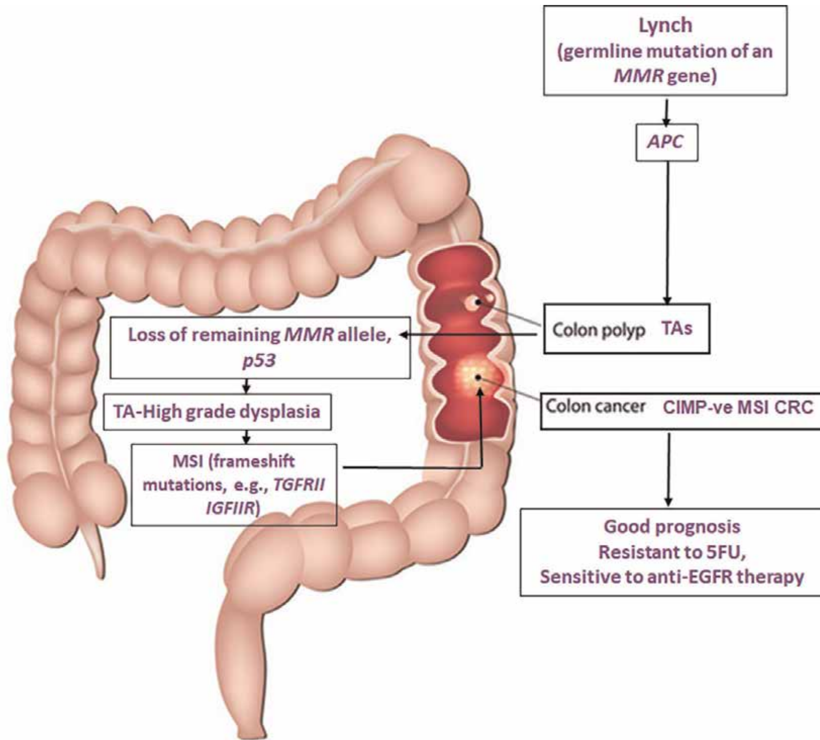

Figure 5.
Hereditary nonpolyposis colorectal cancer (HNCC)/Lynch syndrome pathway to colorectal cancer (CRC). The figure shows the relationship of HNCC pathway lesions to CRC prognosis and therapeutic response. TA: tubular adenoma; IGFIIR, *insulin-like growth factor receptor II;* TGFRβII, *TGF-β receptor II; CIMP-ve: CpG island methylator phenotype-negative; MSI: microsatellite instability; anti-EGFR: epidermal growth factor receptor inhibitors; 5-FU: 5-fluorouracil therapy.*

4.3 Other syndromes predisposing to colorectal cancers

A list of other highly penetrant CRC syndrome cases, with characteristic features and defective genes, is provided in **Table 3**.

4.4 Management of colorectal cancers

In stable economies, death rates for colon cancer have begun to fall, because of early detection and surgical removal of growths that have advanced through only the early stages of tumor progression [77].

5. Familial retinoblastoma

One form of retinoblastoma is hereditary, and the other is not. The hereditary form of retinoblastoma is autosomal dominant and is passed on from one generation to the next according to Mendelian rules of inheritance. The familial form of retinoblastoma occurs in childhood (**Figure 6**). The frequency is 1:20,000 children. In the hereditary form, multiple tumors usually arise independently, affecting both eyes. The neural precursor cells in the immature retina develop tumors. Cytogenetic analysis of a few patients shows a

Sr. no.	Syndrome	Common features	Gene defect
1.	Gardner syndrome	Multiple adenomatous polyps (>100) and carcinomas of the colon and rectum; duodenal polyps and carcinomas; fundic gland polyps in the stomach; congenital hypertrophy of retinal pigment epithelium (CHRPE); desmoid tumors and mandibular osteomas	*APC*
2.	Turcot syndrome	Polyposis and colorectal cancer with brain tumors (medulloblastoma)	*APC*
3.	Colorectal cancer and brain tumors	Colorectal cancer without polyposis and brain tumors (glioblastoma)	*MLH1, PMS2*
4.	Attenuated adenomatous polyposis coli (AAPC)	Fewer than 100 polyps, though marked variation in polyp number (from 5 to >1000 polyps) is seen in mutation carriers within a single family	*APC* (predominantly 5′ mutations)
5.	Peutz-Jeghers syndrome	Hamartomatous polyps throughout the gastrointestinal tract; mucocutaneous pigmentation; estimated 9- to 13-fold increased risk of gastrointestinal (GI) and non-GI cancers	*LKB1/STK11*
6.	Cowden disease	Multiple hamartomas involving breast, thyroid, skin, central nervous system (CNS), and GI tract; increased risk of breast, uterus, and thyroid cancer; risk of GI cancer unclear	*PTEN*
7.	Juvenile polyposis syndrome	Multiple hamartomatous/juvenile polyps with predominance in colon and stomach; variable increase in colorectal and stomach cancer risk; facial changes	*DPC4* *BMPR1A* *PTEN*
8.	Multiple adenoma and colorectal cancer	Multiple colorectal adenomas and colorectal cancer; endometrial cancer and brain tumors in some individuals and families	*POLD1, POLE*
9.	Hereditary mixed polyp syndrome	Multiple types of colorectal polyps (e.g., Peutz-Jeghers polyps, juvenile polyps, serrated lesions, conventional adenomas) and colorectal cancer	*GREM1*

*References are provided in **Table 1**.

Table 3.
Syndromes presenting with colorectal cancers and the underlying gene defects.*

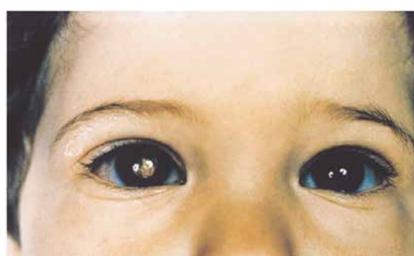

Figure 6.
A pediatric patient with retinoblastoma. Source: NCI (Artist). Retinoblastoma. National Cancer Institute. https://visualsonline.cancer.gov/details.cfm?imageid=2418.

visibly abnormal karyotype, with a deletion of a specific band on chromosome 13. When inherited, the individual has a high risk of the disease. The same deletion was also observed in the tumor cells from some patients with the nonhereditary disease. Chromosomal mapping of the deleted region led to the identification of the *Rb* gene, which is a tumor suppressor. The cancerous retinal cells are defective in both copies of *Rb* because of a somatic event, which makes the previously good copy nonfunctional.

5.1 Translational implications

The cure of the eye tumors can be achieved with radiation or surgery. However, these children are not protected from a highly increased risk (>500 times above normal) of bone cancers (osteosarcomas) during adolescence. In addition, they also have an elevated susceptibility to develop other tumors later in life. Thus, regular surveillance is recommended [78].

6. Von Hippel-Lindau (VHL) syndrome

This is an autosomal dominant disorder and with incidence ranging from 1 in 45,500 to 1 in 36,000 live births [79]. It is a multisystem disorder which predisposes to a variety of malignant and benign neoplasms, most frequently retinal angiomas, cerebellar, spinal cord, and brain stem hemangioblastoma, renal cell carcinoma (RCC), pheochromocytoma, pancreatic tumors, and occasionally extra-adrenal paragangliomas. The condition has been subdivided into three categories: type 1 is associated with renal cell cancer and CNS hemangioblastomas but no pheochromocytoma; type 2 has all of type 1 tumors plus pheochromocytoma. Type 2 can be further subdivided into type 2A which has a low risk of RCC, type 2B which has a high risk of RCC, and type 2C which has just pheochromocytoma [80]. The penetrance of the disease is around 90% by 60 years of age [81].

Over 150 mutations have been identified in VHL all three exons [82]. Approximately 60% of mutations are nonsense and missense mutations, whereas the remainder are whole gene or partial gene deletions. A combination of direct sequencing for the detection of nonsense and missense mutations, plus either Southern blotting, quantitative fluorescent PCR analysis, or MLPA for the detection of large rearrangements, is capable of detecting close to 100% of mutations. Many mutations identified are unique to different families, though a few common mutations are seen, such as that at codon 238 [83].

Certain genotype-phenotype correlations have been reported. A major phenotypic difference between VHL families is the presence or absence of pheochromocytomas. Nearly all families without pheochromocytomas (type 1) have deletions or nonsense mutations [84]. The presence of pheochromocytomas is associated in >90% of cases with missense mutations, mainly the mutation at codon 167. Thus, evidence suggests that the mutant protein must be full length in pheochromocytomas. The codon 169 mutation resulting in tyrosine to histidine substitution has been associated with a highly penetrant form of *VHL*; however, the risk for mortality is not significantly as compared to the general population. Up to 50% of families with only pheochromocytoma have been shown to carry *VHL* mutations. Thus, mutation screening is indicated in this group. Patients with hemangioblastomas should also be offered *VHL* mutation screening as germline mutations have been reported in up to 5% of isolated cases who are <50 years of age [83].

Drugs for *VHL* gene [24]				
Sr. no.	**Name**	**Status**	**Group**	**Mechanism of action**
1.	Sunitinib	Approved, investigational	Pharma	RTK inhibitor, small molecule, antineoplastic agents, protein kinase inhibitors, kinase inhibitors
2.	Belzutifan	Approved, investigational	Pharma	
3.	Bevacizumab	Approved, investigational	Pharma	Antineoplastic agents, therapeutic antibodies, monoclonal antibody
4.	Everolimus	Approved	Pharma	MTOR inhibitors, immunosuppressive agents, kinase inhibitors, small molecule
5.	Temsirolimus	Approved	Pharma	MTOR inhibitors, antineoplastic agents, protein kinase inhibitors, kinase inhibitors, MTOR inhibitor, small molecule, MTOR inhibitors; antitumor

Table 4.
Potential drugs for patients with mutation(s) in the VHL *gene.*

6.1 Translational applications

Systematic screening and early detection of tumors have been shown to reduce morbidity and mortality. Screening of both affected and at-risk individuals is fairly intensive involving annual examinations, direct and indirect ophthalmoscopy, MRI scans at 3-yearly intervals, annual renal ultrasound scans, and annual 24 h urine collection for vanillylmandelic acid (VMA) (**Table 1**) [14].

Once mutation is identified in the *VHL* gene, the possible treatment options are listed in **Table 4**.

7. Hereditary papillary renal cancers

In case of multiple, bilateral papillary renal tumors, hereditary papillary renal cell carcinomas (HPRCs) are suspected. The transmission pattern is consistent with autosomal dominant inheritance; however, the penetrance is incomplete. Heterozygous germline mutations in the *MET* tyrosine kinase gene on 7q32 were detected in families with HPRCs. These mutant germline alleles of *MET* usually carry point mutations that cause amino acid substitutions in the tyrosine kinase domain of *Met*. Consequently, there is constitutive, ligand-independent firing by the receptor [84]. The potential drugs for the *MET* oncogene are listed in **Table 5**.

8. Future perspectives

A number of targeted therapies have been listed in **Table 1**. In addition, progress in gene therapy protocols is beginning to pay dividends. The first commercial gene therapy product, Gendicine™, was approved in 2003 by China's State Food and Drug Administration for the treatment of head and neck squamous cell carcinoma. The drug is a recombinant adenovirus vector that delivers wild-type p53 gene [85]. The treatment has proven safe and efficacious, especially in combination with chemo- and

Drugs for *MET* gene [24]					
Sr. no.	**Name**	**Status**	**Group**	**Role**	**Mechanism of action**
1.	Tepotinib	Approved, investigational	Pharma	Inhibitor, target, inhibition	Selective MET inhibitor. (Efficacy in *MET* exon 14 skipping mutations and amplification).
2.	Capmatinib	Approved, investigational	Pharma	Inhibitor, target, inhibition	C-Met inhibitor, ATP-competitive and novel, MET inhibitor, kinase inhibitors
3.	Crizotinib	Approved, investigational	Pharma	Inhibitor, target, inhibition	C-MET/ALK inhibitor, potent and ATP-competitive, kinase inhibitors, small molecule, ALK inhibitor, potent c-MET/ALK inhibitor
4.	Levoleucovorin	Approved, experimental, investigational	Nutra		Folate analog, adjuvant used in combination with the chemotherapy drugs, small molecule, antineoplastic adjuncts, vitamins/minerals
5.	Cabozantinib	Approved, investigational	Pharma	Inhibitor, antagonist, target, inhibition	VEGFR2/Met/Ret/Kit/FLT//AXL inhibitor, kinase inhibitors, small molecule, potent VEGFR inhibitor; also inhibits other RTKs
Tocris compounds for *MET* gene					
Sr. no.	Compound	Action			
1.	Norleual	Highly potent HGF/c-MET inhibitor; also AT4 antagonist			
2.	PF 04217903 mesylate	Highly selective *MET* inhibitor			
3.	PHA 665752	Potent and selective MET inhibitor			
4.	SGX 523	Selective and potent c-MET kinase inhibitor			
5.	SU 11274	Selective inhibitor of MET kinase activity			
ApexBio compounds for *MET* gene					
Sr. no.	Compound	Mechanism of action			
1.	(R)-Crizotinib	C-MET/ALK inhibitor, potent and ATP-competitive			
2.	Altiratinib	c-MET/TIE-2/VEGFR inhibitor			
3.	AMG 337	MET inhibitor			
4.	AMG-208	C-Met inhibitor, potent and highly selective			
5.	AMG-458	Potent c-Met inhibitor			

Table 5.
Potential inhibitors for patients with mutation(s) in MET *oncogene.*

radiotherapy. As of 2023, more than 30,000 people have been treated with Gendicine™ [86].

In 2007, another gene therapy product, Rexin-G, was approved by regulatory authorities in the Philippines for the treatment of all solid tumors [85]. It carries mutant cytocidal gene cyclin D1, which leads to apoptosis in cancer cells. Rexin-G, the first tumor-targeting injectable therapy, has been approved by the US-FDA for the treatment of metastatic pancreatic cancer [87].

At present, 12 gene therapies have been approved by the US-FDA, European Medicines Agency (EMA), and China State Food and Drug Administration (CFDA) for the treatment of various cancers [88]. In addition, clinical trials utilizing gene editing tools based on clustered regularly interspersed palindromic sequences (CRISPR)-Cas9 technology are also in progress [89].

The penetrance of germline variations in cancers remains to be quantified due to reported discrepancies. Thus, consortia with large sample sizes rather than single-center studies are recommended. The development of resistance to targeted therapies, due to the evolutionary nature of cancers needs to be investigated urgently. In this context, the results and analysis of clinical trials such as Molecular Analysis for Therapy Choice (MATCH) [90], Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging And moLecular Analysis 2 (I-SPY2-TRIAL) [91], BEAT-cc (Atezolizumab plus bevacizumab and chemotherapy for metastatic, persistent, or recurrent cervical cancer (BEATcc): a randomized, open-label, phase 3 trial [92] and the Actionable Genome Panel Consortium (AGP) [6] are eagerly awaited. However, the preponderance of enrolled subjects belongs to Western Europe and some East Asian communities, thus these studies need to be conducted in other populations for validation.

9. Conclusions

In the last two decades, precision medicine has come off age, and the field of oncology now benefits from targeted therapies with fewer side effects than ever before. The study of hereditary cancers has led to a revolution in the management of cancers in general. The premise is that molecular elucidation of cancers can lead to better disease management. Apart from prevention and treatment strategies based on targeted therapies, such therapeutic measures can also aid in conventional cancer therapies, including chemo- and radiotherapy. Survival rates have improved as the molecular pathologies of investigated cancers have been delineated. Translational applications have not only been restricted to cancers but other aspects of health, such as embryogenesis and developmental disorders.

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Cancer researchers worldwide, families, and patients with hereditary cancers contributing to the eradication of disease one step at a time through enrollment in research studies.

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Conflict of interests

None.

Abbreviations

5-FU	5-fluorouracil
AG	actionable genome panel consortium
APC	adenomatous polyposis coli
AT	ataxia telangiectasia
BCT	breast conserving therapy
CDH1	cadherin 1 (E-Cadherin) gene
CFDA	China state food and drug administration
CHRPE	congenital hypertrophy of retinal pigment epithelium
CIMP-ve	CpG island methylator phenotype-negative
COX	cyclooxygenase
CRC	colorectal cancer
CRISPR	clustered regularly interspersed palindromic sequences
EGFR	epidermal growth factor receptor
EMA	European medicines agency
FANCN	fanconi anemia, complementation group N
FAP	familial adenomatous polyposis
FIGC	familial intestinal gastric cancer
GAPPS	gastric adenocarcinoma with proximal polyposis of the stomach
GIST	gastrointestinal stromal tumor
HBOC	hereditary breast and ovarian cancers
HDGC	hereditary diffuse type gastric cancer
HER2	human epidermal growth factor receptor 2
HNCC	hereditary nonpolyposis colorectal cancer
ICC	interstitial cells of Cajal
IGFIIR	insulin-like growth factor receptor II
I-SPY2-TRIAL	investigation of serial studies to predict your therapeutic response with imaging and molecular analysis 2
MATCH	molecular analysis for therapy choice
MEN	multiple endocrine neoplasia
MMR	mismatch repair
MSI	microsatellite instability
MTC	medullary thyroid cancer
mTOR	mammalian target of Rapamycin
NF2	neurofibromin 2
PARP	polyADP-ribose polymerase
PDGFR	platelet-derived growth factor receptor
PTC	papillary thyroid carcinoma
PTEN	phosphatase and tensin homolog
RCC	renal cell carcinoma
RET	rearranged during transfection
SDH	succinyl dehydrogenase
TA	tubular adenoma
US-FDA	United States-Food and Drug Administration
VEGFR	vascular endothelial growth factor receptor
VHL	Von Hippel Lindau
VMA	vanillylmandelic acid

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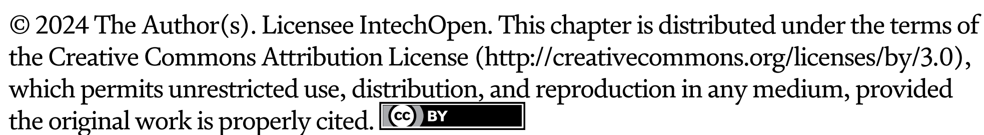
Author details

Sadia Ajaz
Department of Medical Education and International Linkages, University of Health Sciences, Lahore, Pakistan

*Address all correspondence to: sadiaajaz1@gmail.com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References

[1] Broca P. Traite des Tumerurs. Paris, France: P. Asselin; 1866

[2] Banks W. The Lettsomian lectures being practical observations on cancer of the breast: Delivered before the medical society of London. BMJ. 1900;**1**:685-690

[3] Richardson MH. Cancer of the breast. Journal of the American Medical Association. 1909;**52**:1553-1557

[4] Stadler ZK, Shah S, Offit K. In: Gelmann EP, Sawyers CL, Rauscher FJ III, editors. Molecular Oncology. Cambridge, United Kingdom: Cambridge University Press; 2014. pp. 10-20

[5] Alberts B, Heald R, Johnson A, Morgan D, Raff M, Roberts K, et al. Chapter 20 cancer. In: Molecular Biology of the Cell: Seventh International Student Edition with Registration Card. New York: WW Norton & Company; 2022. pp. 1163-1216

[6] Fouad YA, Aanei C. Revisiting the hallmarks of cancer. American Journal of Cancer Research. 2017;**7**(5):1016-1036

[7] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;**144**(5):646-674. DOI: 10.1016/j.cell.2011.02.013

[8] Tsaousis GN, Papadopoulou E, Apessos A, Agiannitopoulos K, Pepe G, Kampouri S, et al. Analysis of hereditary cancer syndromes by using a panel of genes: Novel and multiple pathogenic mutations. BMC Cancer. 2019;**19**(1):535. DOI: 10.1186/s12885-019-5756-4

[9] Weinberg R. Chapter 05 growth factors, receptors, and cancer. In: Biology of Cancer. Garland: Taylor and Francis; 2014. pp. 131-174

[10] Kuhlen M, Taeubner J, Brozou T, Wieczorek D, Siebert R, Borkhardt A. Family-based germline sequencing in children with cancer. Oncogene. 2019;**38**(9):1367-1380. DOI: 10.1038/s41388-018-0520-9. Epub 2018 Oct 10

[11] Yurgelun MB, Hampel H. Recent advances in Lynch syndrome: Diagnosis, treatment, and cancer prevention. American Society of Clinical Oncology Educational Book. 2018;**38**:101-109. DOI: 10.1200/EDBK_208341

[12] eviQ Cancer Treatments Online. 2023. APC (Familial adenomatous polyposis) – risk management. ID: 178 v. 9 [Accessed: November 28, 2023]

[13] Kemp Bohan PM, Mankaney G, Vreeland TJ, Chick RC, Hale DF, Cindass JL, et al. Chemoprevention in familial adenomatous polyposis: Past, present and future. Familial Cancer. 2021;**20**(1): 23-33. DOI: 10.1007/s10689-020-00189-y. Epub 2020 Jun 8

[14] eviQ Cancer Treatments Online. 2023. VHL (von Hippel-Lindau) disease – risk management. ID: 397 v.9 [Accessed: November 29, 2023]

[15] Belzutifan Approved to Treat Tumors Linked to Inherited Disorder VHL was originally published by the National Cancer Institute. Available from: https://www.cancer.gov/news-events/cancer-currents-blog/2021/fda-belzutifan-vhl-tumors [Accessed: November 29, 2023]

[16] Salgia R, Sattler M, Scheele J, Stroh C, Felip E. The promise of selective MET inhibitors in non-small cell lung cancer with MET exon 14 skipping. Cancer Treatment Reviews. 2020;**87**:102022. DOI: 10.1016/j.ctrv.2020.102022. Epub 2020 Apr 9

[17] eviQ Cancer Treatments Online (2023). CDKN2A – risk management ID: 1516 v.5 [Accessed: November 30, 2023]

[18] Adib E, Nassar AH, Akl EW, Abou Alaiwi S, Nuzzo PV, Mouhieddine TH, et al. *CDKN2A* alterations and response to immunotherapy in solid tumors. Clinical Cancer Research. 2021;**27**(14):4025-4035. DOI: 10.1158/1078-0432.CCR-21-0575. Epub 2021 Jun 1

[19] World Health Organization (WHO). Available from: https://www.who.int/news-room/fact-sheets/detail/colorectal-cancer [Accessed: December 15, 2023]

[20] Bhandari J, Thada PK, Puckett Y. Fanconi Anemia. Treasure Island (FL): StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559133/

[21] National Comprehensive Cancer Network® (NCCN®) Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment: Breast, Ovarian and Pancreatic v.1. 2023. National Comprehensive Cancer Network, Inc; 2022. Available from: www.nccn.org [Accessed: October 24, 2022]

[22] eviQ Cancer Treatments Online. 2023. *PALB2* – risk management. ID: 1609 v.8 [Accessed: November 30, 2023]

[23] Weinberg R. Chapter 12. Maintenance of genomic integrity and the development of cancer. In: Biology of Cancer. Garland: Taylor and Francis; 2014. pp. 511-574

[24] Genecards. Available from: https://www.genecards.org/ [Accessed: November 30, 2023]

[25] eviQ Cancer Treatments Online. 2023. ATM (monoallelic pathogenic variants) – risk management ID: 1610 v.9 [Accessed: December 1, 2023]

[26] Evaluation of a Promising New Combination of Protein Kinase Inhibitors on Organotypic Cultures of Human Renal Tumors (COMBOREIN): Available from: https://classic.clinicaltrials.gov/ct2/show/NCT03571438 [Accessed: December 1, 2023]

[27] Whiteaker JR, Zhao L, Saul R, Kaczmarczyk JA, Schoenherr RM, Moore HD, et al. A multiplexed mass spectrometry-based assay for robust quantification of phosphosignaling in response to DNA damage. Radiation Research. 2018;**189**(5):505-518. DOI: 10.1667/RR14963.1. Epub 2018 Feb 23

[28] Cunniff C, Djavid AR, Carrubba S, Cohen B, Ellis NA, Levy CF, et al. Health supervision for people with bloom syndrome. American Journal of Medical Genetics. Part A. 2018;**176**(9):1872-1881. DOI: 10.1002/ajmg.a.40374. Epub 2018 Jul 28

[29] Leung AK, Barankin B, Lam JM, Leong KF, Hon KL. Xeroderma pigmentosum: An updated review. Drugs Context. 2022;**11**:2022-22-5. DOI: 10.7573/dic.2022-2-5

[30] Tsujimoto M, Kakei Y, Yamano N, Fujita T, Ueda T, Ono R, et al. Clinical trial on the efficacy and safety of NPC-15 for patients with xeroderma pigmentosum exaggerated sunburn reaction type: XP-1 study protocol for a multicentre, double-blinded, placebo-controlled, two-group crossover study followed by a long-term open study in Japan. BMJ Open. 2023;**13**(3):e068112. DOI: 10.1136/bmjopen-2022-068112

[31] Ahmad SB, Ali A, Bilal M, Rashid SM, Wani AB, Bhat RR, et al. Melatonin and health: Insights of melatonin action,

biological functions, and associated disorders. Cellular and Molecular Neurobiology. 2023;**43**(6):2437-2458. DOI: 10.1007/s10571-023-01324-w. Epub 2023 Feb 8

[32] Hasbaoui BE, Elyajouri A, Abilkassem R, Agadr A. Nijmegen breakage syndrome: Case report and review of literature. The Pan African Medical Journal. 2020;**35**:85. DOI: 10.11604/pamj.2020.35.85.14746

[33] Mahamdallie S, Yost S, Poyastro-Pearson E, Holt E, Zachariou A, Seal S, et al. Identification of new Wilms tumour predisposition genes: An exome sequencing study. The Lancet Child & Adolescent Health. 2019;**3**(5):322-331. DOI: 10.1016/S2352-4642(19)30018-5. Epub 2019 Mar 16

[34] Balis F, Green DM, Anderson C, Cook S, Dhillon J, Gow K, et al. Wilms tumor (Nephroblastoma), version 2.2021, NCCN clinical practice guidelines in oncology. Journal of the National Comprehensive Cancer Network. 2021;**19**(8):945-977. DOI: 10.6004/jnccn.2021.0037

[35] World Health Organization (WHO). Available from: https://www.who.int/news-room/fact-sheets/detail/breast-cancer [Accessed: December 18, 2023]

[36] Gaona-Luviano P, Medina-Gaona LA, Magaña-Pérez K. Epidemiology of ovarian cancer. Chinese Clinical Oncology. 2020;**9**(4):47. DOI: 10.21037/cco-20-34. Epub 2020 Jun 30

[37] Yoshida R. Hereditary breast and ovarian cancer (HBOC): Review of its molecular characteristics, screening, treatment, and prognosis. Breast Cancer. 2021;**28**(6):1167-1180. DOI: 10.1007/s12282-020-01148-2. Epub 2020 Aug 29

[38] Mucci LA, Hjelmborg JB, Harris JR, Czene K, Havelick DJ, Scheike T, et al. Familial risk and heritability of cancer among twins in Nordic countries. JAMA. 2016;**315**(1):68-76. DOI: 10.1001/jama.2015.17703. Erratum in: JAMA. 2016 Feb 23;315(8):822

[39] Cancer net. Available from: https://www.cancer.net/cancer-types/hereditary-breast-and-ovarian-cancer [Accessed: December 1, 2023]

[40] Center for Disease Control (CDC). Available from: https://www.cdc.gov/genomics/implementation/toolkit/hboc_patient_info.htm [Accessed: December 1, 2023]

[41] Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994;**266**:66-71

[42] Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene *BRCA2* [see comments]. Nature. 1995;**378**:789-792. Published erratum appears in Nature 1996; 379:749

[43] Cornelis RS, Neuhausen SL, Johansson O, et al. High allele loss rates at 17q12-21 in breast and ovarian tumors from BRCA1-linked families. The breast cancer linkage consortium. Genes, Chromosomes & Cancer. 1995;**13**:203-210

[44] Collins N, McManus R, Wooster R, et al. Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12-13. Oncogene. 1995;**10**:1673-1675

[45] Moyer VA, US Preventive Services Task Force. Risk assessment, genetic counseling, and genetic testing for *BRCA*-related cancer in women: US

preventive services task force recommendation statement. Annals of Internal Medicine. 2014;**160**(4):271-281

[46] Domchek SM, Friebel TM, Singer CF, et al. Association of risk-reducing surgery in *BRCA1* or *BRCA2* mutation carriers with cancer risk and mortality. Journal of the American Medical Association. 2010;**304**(9):967-975

[47] King MC, Levy-Lahad E, Lahad A. Population-based screening for BRCA1 and BRCA2: 2014 Lasker award. Journal of the American Medical Association. 2014;**312**(11):1091-1092. DOI: 10.1001/jama.2014.12483

[48] National Cancer Institute (NCI). FDA Approves Rucaparib for BRCA-Positive Ovarian Cancer was originally published by the National Cancer Institute. Available from: https://www.cancer.gov/news-events/cancer-currents-blog/2017/fda-rucaparib-ovarian [Accessed: January 18, 2024]

[49] Malkin D, Li FP, Strong LC, et al. Germline p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science. 1990;**250**:1233-1238

[50] Sokolova A, Johnstone KJ, McCart Reed AE, Simpson PT, Lakhani SR. Hereditary breast cancer: Syndromes, tumour pathology and molecular testing. Histopathology. 2023;**82**(1):70-82. DOI: 10.1111/his.14808. Epub 2022 Dec 5

[51] McBride KA, Ballinger ML, Killick E, et al. Li–Fraumeni syndrome: Cancer risk assessment and clinical management. Nature Reviews. Clinical Oncology. 2014;**11**:260-271

[52] Mai PL, Best AF, Peters JA, et al. Risks of first and subsequent cancers among TP53 mutation carriers in the National Cancer Institute Li–Fraumeni syndrome cohort. Cancer. 2016;**122**:3673-3681

[53] Olivier M, Goldgar DE, Sodha N, Ohgaki H, Kleihues P, Hainaut P, et al. Li–Fraumeni and related syndromes: Correlation between tumor type, family structure, and TP53 genotype. Cancer Research. 2003;**63**(20):6643-6650

[54] Schon K, Tischkowitz M. Clinical implications of germline mutations in breast cancer: TP53. Breast Cancer Research and Treatment. 2018;**167**(2):417-423. DOI: 10.1007/s10549-017-4531-y. Epub 2017 Oct 16

[55] Villani A, Shore A, Wasserman JD, Stephens D, Kim RH, Druker H, et al. Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study. The Lancet Oncology. 2016;**17**(9):1295-1305. DOI: 10.1016/S1470-2045(16)30249-2. Epub 2016 Aug 5

[56] Familial breast cancer: classification, care and managing breast cancer and related risks in people with a family history of breast cancer. 1-Recommendations. Guidance and guidelines. NICE. Available from: https://www.nice.org.uk/guidance/cg164/chapter/1-recommendations [Accessed: December 1, 2016]

[57] National Comprehensive Cancer Network. Genetic/familial high risk assessment: Breast and ovarian. Li Fraumeni syndrome management. In: NCCN Clinical Practice Guidelines in Oncology; Version 2.2014. National Comprehensive Cancer Network, Inc; 2022. Available from: www.nccn.org

[58] eviQ Cancer Treatments Online. 2023. TP53 (Li–Fraumeni syndrome) – risk management (adult). ID: 749 v.12 [Accessed: November 24, 2023]

[59] Molière S, Mathelin C. The Cowden syndrome. New England Journal of Medicine. 2020;**382**(15):e29. DOI: 10.1056/nejmicm1910478

[60] Ngeow J, Sesock K, Eng C. Breast cancer risk and clinical implications for germline PTEN mutation carriers. Breast Cancer Research and Treatment. 2017; **165**(1):1-8. DOI: 10.1007/s10549-015-3665-z. Epub 2015 Dec 23

[61] Yari K, Hakimi A, Mohammadi M, Ammari-Allahyari M, Salari N, Ghasemi H. The association of PTEN gene mutations with the breast cancer risk: A systematic review and meta-analysis. Biochemical Genetics. 2023;**62**:1-19

[62] Tischkowitz M, Colas C, Pouwels S, Hoogerbrugge N, PHTS Guideline Development Group. European reference network GENTURIS. Cancer surveillance guideline for individuals with PTEN hamartoma tumour syndrome. European Journal of Human Genetics. 2020;**28**(10):1387-1393. DOI: 10.1038/s41431-020-0651-7. Epub 2020 Jun 12

[63] Wu M, Krishnamurthy K. Peutz-Jeghers Syndrome. Treasure Island (FL): StatPearls Publishing; 2023

[64] OMIM. Available from: https://omim.org/entry/175200#moleculargenetics [Accessed: November 27, 2023]

[65] Okur V, Chung WK. The impact of hereditary cancer gene panels on clinical care and lessons learned. Cold Spring Harbor Molecular Case Studies. 2017;**3**(6):a002154. DOI: 10.1101/mcs.a002345

[66] Price KS, Svenson A, King E, Ready K, Lazarin GA. Inherited cancer in the age of next-generation sequencing. Biological Research for Nursing. 2018;**20**(2):192-204

[67] Kuiper RP, Weren RDA, van Kessel AG. Chapter 11 - colorectal cancer. In: David SP, editor. Genomic and Precision Medicine. 3rd ed. Academic Press, Elsevier, inc; 2017. pp. 195-209. ISBN 9780128006856. DOI: 10.1016/B978-0-12-800685-6.00011-4

[68] Ford JM, Kastan MB. DNA damage response pathways and cancer. In: Niederhuber JE, Armitage JO, Doroshow J, editors. Abeloff's Clinical Oncology. Philadelphia, PA: Churchill Livingstone; 2014. pp. 142-153

[69] Half E, Bercovich D, Rozen P. Familial adenomatous polyposis. Orphanet Journal of Rare Diseases. 2009; **4**:22. DOI: 10.1186/1750-1172-4-22

[70] Weinberg R. Chapter 07. Tumour suppressor genes. In: Biology of Cancer. Garland: Taylor and Francis; 2014. pp. 231-274

[71] Rofes P, González S, Navarro M, Moreno-Cabrera JM, Solanes A, Darder E, et al. Paired somatic-germline testing of 15 polyposis and colorectal cancer-predisposing genes highlights the role of APC mosaicism in de novo familial adenomatous polyposis. The Journal of Molecular Diagnostics. 2021;**23**(11): 1452-1459. DOI: 10.1016/j.jmoldx.2021.07.024. Epub 2021 Aug 25

[72] Leoz ML, Carballal S, Moreira L, Ocaña T, Balaguer F. The genetic basis of familial adenomatous polyposis and its implications for clinical practice and risk management. The Application of Clinical Genetics. 2015;**8**:95-107. DOI: 10.2147/TACG.S51484

[73] GeneCards. Available from: https://www.genecards.org/cgi-bin/carddisp.pl?gene=MUTYH&keywords=MUTHY [Accessed: January 19, 2024]

[74] Liska D, Kalady MF. Colorectal surgery in Lynch syndrome patients:

When and how? Current Colorectal Cancer Reports. 2015;**11**:45-53

[75] Lynch HT, Kimberling W, Albano WA, Lynch JF, Biscone K, Schuelke GS, et al. Hereditary nonpolyposis colorectal cancer (Lynch syndromes I and II). I. Clinical description of resource. Cancer. 1985;**56**:934-938

[76] Imyanitov EN, Kuligina ES, Sokolenko AP, Suspitsin EN, Yanus GA, Iyevleva AG, et al. Hereditary cancer syndromes. World Journal of Clinical Oncology. 2023;**14**(2):40-68. DOI: 10.5306/wjco.v14.i2.40

[77] Rawla P, Sunkara T, Barsouk A. Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors. Przeglad Gastroenterologiczny. 2019;**14**(2):89-103. DOI: 10.5114/pg.2018.81072. Epub 2019 Jan 6

[78] eviQ Cancer Treatments Online (2024). RB1 (non-retinoblastoma cancer risk) – risk management. ID: 3704 v.2. [Accessed: January 19, 2024]

[79] Ma X, Tan Z, Zhang Q, Ma K, Xiao J, Wang X, et al. VHL Ser65 mutations enhance HIF2α signaling and promote epithelial-mesenchymal transition of renal cancer cells. Cell & Bioscience. 2022;**12**(1):52. DOI: 10.1186/s13578-022-00790-x

[80] Gläsker S, Neumann HPH, Koch CA, Vortmeyer A. Von Hippel-Lindau disease. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, et al, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000

[81] Rana HQ, Koeller DR, Schwartz A, Manning DK, Schneider KA, Krajewski KM, et al. Pathogenicity of VHL variants in families with non-syndromic von Hippel-Lindau phenotypes: An integrated evaluation of germline and somatic genomic results. European Journal of Medical Genetics. 2021;**64**(12):104359. DOI: 10.1016/j.ejmg.2021.104359. Epub 2021 Oct 8

[82] UMD. Available from: www.umd.necker.fr.2005

[83] Macdonald F, Ford C, Casson A. Chapter 5. Hereditary cancers. In: Molecular Biology of Cancer. New York: Garland Science/BIOS Scientific Publishers; 2004

[84] Online Mendelian Inheritance in Man (OMIM). Available from: https://www.omim.org/entry/605074 [Accessed: January 19, 2024]

[85] Lundstrom K. Viral vectors in gene therapy: Where do we stand in 2023? Viruses. 2023;**15**(3):698. DOI: 10.3390/v15030698

[86] Shahryari A, Saghaeian Jazi M, Mohammadi S, Razavi Nikoo H, Nazari Z, Hosseini ES, et al. Development and clinical translation of approved gene therapy products for genetic disorders. Frontiers in Genetics. 2019;**10**:868. DOI: 10.3389/fgene.2019.00868

[87] Thoidingjam S, Sriramulu S, Freytag S, Brown SL, Kim JH, Chetty IJ, et al. Oncolytic virus-based suicide gene therapy for cancer treatment: A perspective of the clinical trials conducted at Henry Ford Health. Translational Medicine Communications. 2023;**8**(1):11. DOI: 10.1186/s41231-023-00144-w. Epub 2023 Apr 10

[88] National Cancer Institute. Available from: https://www.cancer.gov/news-events/cancer-currents-blog/2020/crispr-cancer-research-treatment [Accessed: January 22, 2024]

[89] Subbiah V, Burris HA 3rd, Kurzrock R. Revolutionizing cancer drug development: Harnessing the potential of basket trials. Cancer. 2024;**130**(2): 186-200. DOI: 10.1002/cncr.35085. Epub 2023 Nov 7

[90] Albain KS, Yau C, Petricoin EF, Wolf DM, Lang JE, Chien AJ, et al. Neoadjuvant Trebananib plus paclitaxel-based chemotherapy for stage II/III breast cancer in the adaptively randomized I-SPY2 trial-efficacy and biomarker discovery. Clinical Cancer Research. 2024;**30**(4):729-740. DOI: 10.1158/1078-0432.CCR-22-2256

[91] Oaknin A, Gladieff L, Martínez-García J, Villacampa G, Takekuma M, De Giorgi U, et al. ENGOT-Cx10–GEICO 68-C–JGOG1084–GOG-3030 investigators. Atezolizumab plus bevacizumab and chemotherapy for metastatic, persistent, or recurrent cervical cancer (BEATcc): A randomised, open-label, phase 3 trial. Lancet. 2024;**403**(10421):31-43. DOI: 10.1016/S0140-6736(23)02405-4. Epub 2023 Dec 1

[92] Li BT, Janku F, Jung B, Hou C, Madwani K, Alden R, et al. Ultra-deep next-generation sequencing of plasma cell-free DNA in patients with advanced lung cancers: Results from the actionable genome consortium. Annals of Oncology. 2019;**30**(4):597-603. DOI: 10.1093/annonc/mdz046

Chapter 3

Novel Bio-Engineering Techniques for Construction of Next-Generation Monoclonal Antibodies in the Framework of Personalized Medicine

Mahdi Barazesh, Shiva Mohammadi and Sajad Jalili

Abstract

Monoclonal antibodies (mAbs), belonging to the IgG subclass, are the most progressively growing biopharmaceutics with successful applications for the remediation of chronic disorders, including tumors, inflammatory diseases, and retinal neovascularization. Several engineered platforms have been developed recently for the construction of new generations of these recombinant proteins with improved affinity, improved antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) function, improved effector activity, and therapeutic properties. These include engineered antibody fusion proteins such as CAR-T and CAR-NK cells, immunotoxin, bispecific antibodies, antibody-cytokine, and drug-conjugated antibodies. This chapter discusses various bioengineering techniques, focusing on genetic and protein engineering methods. Besides, it describes the superiority and drawbacks of these technologies for monoclonal antibody production by considering stability, effectiveness, affinity, and bio-safety for human applications. There is still potential for developing a novel technique that is straightforward, rapid, and affordable while ensuring the stability and efficiency of these biotherapeutic agents in the framework of personalized medicine. Contribution of these novel techniques to develop antibodies for personalized medicine application is anticipated through the quick generation of individual-specific antibodies with better therapeutic efficacy, affinity, and stability as well as the development of combinatorial medications and innovative antibody delivery platforms.

Keywords: genetic engineering, improved affinity maturation, naive antibody gene library, personalized medicine, protein engineering, ribosome display

1. Introduction

Drug products are a group of macromolecules with therapeutic, diagnostic applications that are purified or produced from a living source and also named as

biotherapeutics or biologics [1]. Therapeutic products such as growth factors, vaccines, cytokines, hormones, recombinant proteins, medicinal peptides, and antibody-drug conjugates (ADCs) belong to this group. Monoclonal antibodies (mAbs) are the most quickly evolving biotherapeutics utilized prosperously to remedy chronic disorders, including tumors, inflammatory diseases, and optical microvascularization [2]. IgG subclass of antibodies is mono-specific, bivalent molecules constituted of a fragment antigen binding (Fabs) domain involved in specific antigen binding or cell receptor recognition and cytokine activation, and a fragment crystallizable (Fc) region is responsible for stability and Fc-mediated recovery, effector functions, and long half-life in circulation [3]. Indeed, each antibody molecule is constituted of two heavy chains (each containing variable (VH), joining (JH), diversity (D), and constant (C) domain) and two light chains (each including variable (VL), joining (JL), and constant (C) domain) that are bounded non-covalently [4]. After being prescribed in the patient, IgG stability preservation is the major concern since its aggregation or degradation leads to unwanted immunogenicity. Thus, to increase the functionality and stability of mAbs for developing novel biotherapeutics, continuous endeavors to design the next generation of therapeutic antibodies are constantly underway using genetic and protein engineering techniques for replacing amino acid residues with higher stability [5].

Antibody mimetics represent a modern approach to antibody engineering. These compounds mimic antibodies by interacting with specific antigens, but they are synthetic peptides and not produced by immune system like real antibodies [6]. They can be developed through protein engineering, designing synthetic peptide, or combining complementarity-determining regions (CDRs) with framework (FR) regions to enhance their recognition capabilities, strong binding interaction, stability, permeability, and affordability [7]. ADCs especially antibody mimetic-drug conjugates (AMDCs), for instance, non-covalent attachment of modified iminobiotin with mutated streptavidin as miniprotein antibodies, are a key treatment option for advanced cancer. Kalbitor (Dyax), affibodies, adnectins, affimers, aptamers, designed ankyrin repeat proteins (DARPin), and knottin molecules, each tailored for certain characteristics like pH resistance, stability against protease degradation, and low immunogenicity are other examples of antibody mimetics [8].

Personalized medicine is leveraging antibody technologies to pinpoint crucial proteins (biomarkers) involved in diseases. By using high-throughput data such as omics technologies and reorganizing genetic structures such as antibody genes, scientists can create novel protein combinations for personalized uses. For instance, in precision medicine, antibody technologies are employed to pinpoint breast cancer patients that respond to trastuzumab treatment [9]. Antibody-based SNAP-tag proteins serve as a precision medicine tool to identify patients likely to respond well to specific treatments with the optimized pharmacokinetic and pharmacodynamics dose and subsequently leading to decreased side effects and better outcomes [10].

In recent years, mAbs have revolutionized clinical backgrounds. Hybridoma technology, the first conventional technique for producing these biotherapeutics, expanded in the middle of the 1970s. In the late 1980s, mAbs generation was initially based on immunized animals, applying laboratory mice, rabbits, and other experimental animal models [11]. The main obstacle in establishing and applying produced mAbs in these animals was ineffective immune stimulation versus highly toxic or particular epitopes. Furthermore, the most utilized mAbs in clinical settings should have human source or are minimally humanized in certain parts to hamper undesired immunogenic response [2]. Thus, to obviate the immunogenicity problem, the animal

models containing antibody transgenes of human origin have been generated without the need for efficient immunogenicity following immunization. Fully humanized mAbs and antibody fragments were also produced experimentally using engineering techniques including display platforms and synthetic biology methods in cell-free-based approaches [12].

Indeed, the emergence of novel molecular techniques has caused rapid growth for production of recombinant mAbs in *in vitro* conditions using different model organisms such as single-cell fungi, microbial systems, and novel platforms for choosing genetically modified recombinant libraries applying different display strategies [13]. Overall, recombinant mAbs technologies aim to obtain antisera containing high-titers, uni-specific, and high-affinity antibodies. Furthermore, therapeutic mAbs have been established by Fc modifications to improve their effector action and site-specific directed glycosylation related to the generation of immunoglobins with improved therapeutic impacts [14]. Approved mAbs by the US Food and Drug Administration (FDA) are rising globally. In other words, about four new products are approved annually. Presently, 47 mAb products in the US, Europe, and worldwide markets have been approved for the treatment of a variety of disorders. At the current speed, approximately 100 mAb products will be on the market by 2025, and overall global commerce will be nearly $150 billion [15].

Up to now, confined comprehension exists on developed perspectives of the antibody generation using the hybridoma technique, antibody engineering methods, manufacturing of fragment versions of antibodies, display strategies, and their wide utilization. Thus, to tackle these health menaces and drawbacks, fantastic progressions in hybridoma technique and recombinant antibody engineering technologies from different aspects, including diagnostics and widespread therapeutic applications, have been reviewed in detail in this chapter. Besides, an overview of possible future progressions for the production of the next generations of mAbs with improved safety and efficacy will be provided.

2. An overview of conventional strategy for mAbs generation

2.1 Hybridoma technique

The Hybridoma platform has been a popularized and classical route for the generation of high-quality specific mAbs in the natural format. However, methodological drawbacks in this technology have directed the antibody generation workflow into novel methods such as display and transgenic mice strategies [16]. The technique starts with immunization of laboratory animals by exposure to a specific antigen during a period of several weeks, and antiserum concentration is measured by immunoassay technique including enzyme-linked immunosorbent assay (ELISA). At the next stage, the splenocytes (activated B lymphocytes) are harvested at sterile conditions and hybridized with myeloma cells to generate hybridoma lines, leading to the permanent generation of a specific monoclonal antibody against a single epitope. Hybridoma cell lines are then propagated in the plate with 96-wells format in the existence of hypoxanthine-aminopterin-thymidine (HAT) conditional media for high-scale selection. Subsequently, hybridoma cell lines with high production capability of interested antibodies are selected by traditional ELISA and novel colloidal gold or silver nanoparticles-probed immunoassay (**Figure 1A**). Specific mAb-producing hybridoma lines *in vitro* then underwent large-scale production by conditional media,

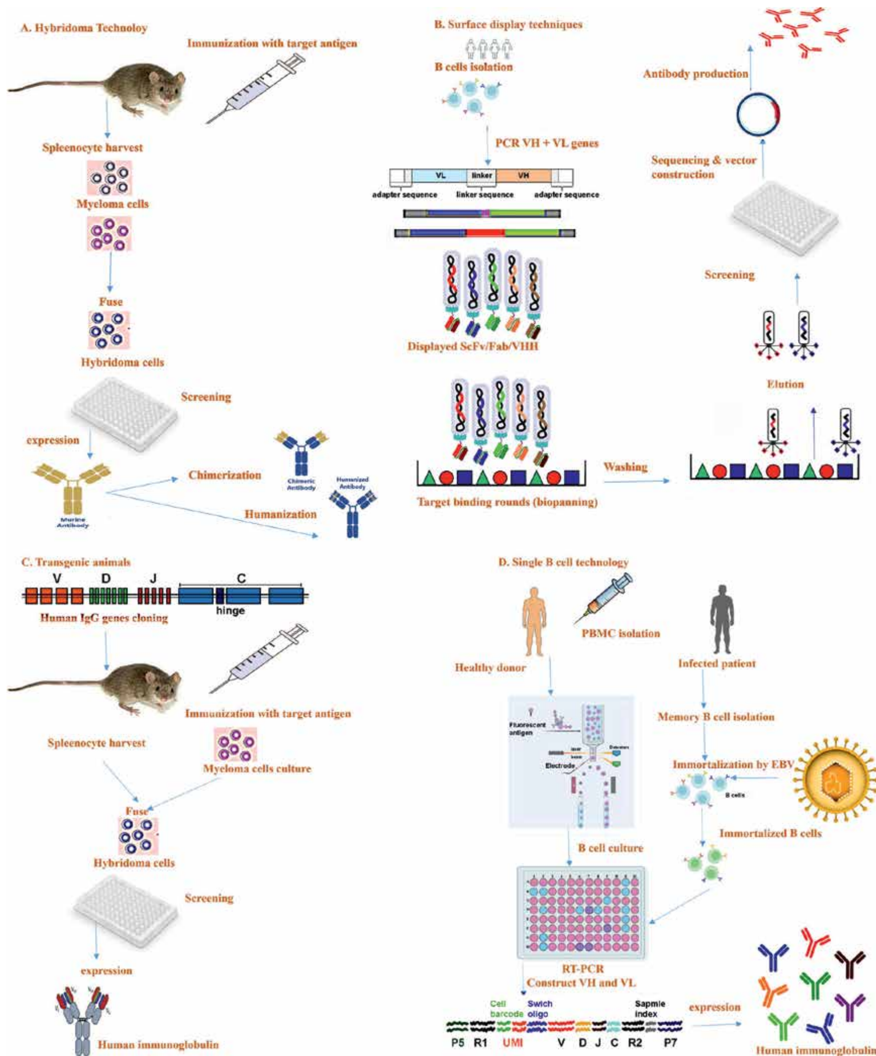

Figure 1.
Different platforms for design and production of next generation mAbs. A. The conventional mouse hybridoma technology initiates via *mouse immunization with interested epitopes to stimulate an immune response. Separated splenocytes are fused with myeloma cells to generate hybridoma cells that constitutively secrete antibodies. After the ElISA analysis, chosen leads are applied to manufacture chimeric or humanized antibodies. B. Phage display. A human phage-displayed human antibody library is utilized to choose target epitopes. Following 3–5 cycles of biopanning, immuno-positive phage clones are analyzed by ELISA; then, DNA sequences are performed to generate and produce human IgGs. C. Transgenic mouse. Like to the mouse hybridoma technology or single B cell approaches. D. The Single B cell technology. From immunized or vaccinated donors, PBMCs are separated for screening of appropriate B cells by flow cytometry. After the RT-PCR, V_H and V_L sequences of each B cell isotype were used for the generation of human mAbs.*

shaker flasks, and bench-scale fermenters under approved good manufacturing practice (cGMP) regulations for clinical applications following pilot scale for scalability and toxicological evaluations. The Hybridoma technique has also been utilized to produce single-chain fragment variable (scFvs) [11].

3. Novel platforms for production of next-generation mAbs

The small-sized versions in form of fragmented antibodies, including Fab, scFv, and nanobodies, are developing biotherapeutic-based medications with better tissue penetration and higher bioavailability. The potency of smaller-sized formats of antibody can be ameliorated by enhancing the efficient concentration with a higher affinity for target recognition. Other benefits of small-sized antibody fragments are higher facility and affordability in terms of their production process compared to mAbs as a result of the lack of specific glycosylation, high penetration ratio into the target tissue, and their production in a simple expression system like bacteria [17]. Four drugs belong to the Fab format approved by the FDA for various clinical settings, including retinal neovascularization and rheumatoid arthritis (RA) [18]. scFv is a novel version of recombinant antibody in which VH and VL domains are fused as single molecule using a flexible linker. To improve stability and higher affinity, amino acid residues in VH and VL are engineered. Hitherto, only one scFv, brolucizumab, has obtained FDA approval (2021) and applied for treating age-related macular degeneration (AMD) [19]. However, post-clinical application problems against biosafety and longevity have been announced to the American Society of Retinal Specialists (ASRS), and patient reports have frequently been published. Thus, there is still a requirement to produce the novel engineered scFv to conquer the obstacles related to longevity and adverse effects [20]. To overcome these limitations, different approaches have been employed to improve the life span of fragment versions and extend their stability, including linking to other proteins (for instance, albumin) or to large molecules (for instance, PEG polymer). For example, an FDA-approved Fab version against TNFa named certolizumab pegol belongs to this category and is applied for treating RA [21].

Various genetic manipulation methods have been established for the production of fragments format with the purpose of enhancing their longevity in circulation and binding affinity. For instance, bispecific T-cell engagers (BiTEs) are manufactured by joining two scFvs (each derived from different monospecific mAbs) *via* a linker. This flexible linker can rotate freely to interact with two distinct target antigen moieties and also help BiTE stability with improved functional and binding affinity compared to a scFv alone. Blinatumomab (obtained its approval in 2016), tebentafusp (2022), and solitomab (currently in clinical trials) belong to BiTEs subtypes and were used for curing acute myeloid leukemia, ocular tumor, and colorectal/lung tumors, respectively [22].

3.1 Bioengineering technologies for the antibody fragments (Fabs and scFvs) manufacturing

Different platforms have been developed for antibody fragment generation, including enzymatic digestion of monoclonal antibodies and recombinant heterologous protein expression systems. Various expression systems, including microbial host (*E. coli*), yeast (*Pichia pastoris*), fungi, insects, mammalian cells such as Chinese hamster ovary (CHO), or even whole organisms such as animals and plants, have been efficiently applied [23]. Although some of these antibody formats were initially purified from human sources in approximately low amounts, mass production of these biotherapeutics nowadays is performed by recombinant technologies using cell-based expression systems and has been discussed further in the next sections.

3.1.1 In vivo display techniques using phage, bacteria, yeasts, and mammalian systems

Display approach using a phage system was applied for production of the first mAb of human origin, adalimumab, using genetically engineered bacteriophage and repetition cycles of antigen-directed selection and phage propagation. Phage libraries were constructed by amplification of all transcribed recombined variable domains composed of all possible variable heavy and light chains within a certain immunoglobulin repertoire. The constructed cDNA libraries are then cloned into phagemid vectors [24]. Rearranged VH and VL repertoires are displayed on the phage surface. Following panning selection, phage carrying the recombinant gene can infect the susceptible *E. coli* strains for propagation of phagemids consisting of certain antibodies or obtaining expressed pure recombinant antibodies. Produced antibodies are evaluated to determine binding affinity and the dynamic interaction against their specific antigens using the surface plasmon resonance (SPR) approach [25]. Other methods to characterize isotype, cross-binding, specificity, and affinity of scFv antibody include phage-ELISA, immunoblotting, immunofluorescence antibody assay, molecular weight spectrometry, sequencing, and nuclear magnetic resonance (NMR) spectroscopy (**Figure 1B**). Various libraries for diverse antibody formats (e.g., scFv, Fv, Fabs versions, diabodies, and bispecific antibodies) could be constructed using the phage display approach. However, the complexity, unaffordability, and time-consuming nature of the technique limits its application. Moreover, the misfolding of heavy and light chains may lead to the formation of nonfunctional antibody fragments [26].

Similar *in vivo* display methods such as bacterial, yeast, and mammalian surface display are also available. Synthetic biology methods along with genetic engineering technologies (for instance, clustered regularly interspaced short palindromic repeats/crispr-associated protein (CRISPR/Cas) tool) have facilitated the establishment of engineered hosts for display applications by changing their natural properties, including modifying cytoplasmic membrane/cell wall components or protein secretory routes. Endeavors related to chaperone engineering and other proteins involved in folding, translocation components, and vesicle trafficking have improved the concomitant secretion of soluble and displayed protein to facilitate their identification [27]. Chelating recombinant antibodies (CRAbs) are another antibody format produced by display technologies. CRAbs are composed of scFv segments with a high binding affinity. This antibody format consists of two distinct scFv domains fused by a small-sized linker for the formation of diabody against the intended antigens [28]. Full IgG with desired post-translation alterations and other engineered antibody fragments have also been produced by different display methods including CDRs, Fab, F(ab')2, monospecific Fab2, bispecific Fab2, tri-specific Fab3, monovalent IgG, bispecific diabody, tri-specific triabody, scFv-Fc, mini body, new antigen receptor (IgNAR), variable new antigen receptor (VNAR) domains in sharks, camelid heavy chain IgG (hcIgG), and VHH fragments [29].

3.1.2 In vitro display techniques applying ribosome and mRNA

Display approaches using ribosome and mRNA are cell-free based techniques and have higher biodiversity of antibody libraries ranging from 10^{12} to 10^{14} clones that facilitate antibody purification with higher affinities at Pico molar (pM) levels for each interested antigen. The fundamental characteristics of both techniques are similar, including efficient *in vitro* transcription and translation procedures. The antibody

DNA library is first transcribed to mRNA using the ribosome-display approach. The ribosome is then attached to *an in vitro* expressed peptide encoded by a library of mRNA-generating antibodies. Next, an affinity chromatography method may be used to select a peptide-ribosome-mRNA (PRM) complex that matches the sequence information of the desired antibody. High diversity of libraries, which are not restricted by the efficiency of cell transformation, is a major benefit of this approach. Conversely, the main obstacles of this approach are ribosomes content and unspecific translated mRNAs [30].

In the mRNA display approach, the antibody DNA library is first transcribed to mRNA. Next, mRNA is bound to a DNA linker sequence fused to puromycin. Afterward, the mRNA-linker-puromycin complex is entered into the A-site of the ribosome by puromycin and then located the P-site, and the newly expressed peptide is transferred to puromycin. Consequently, the mRNA-linker-puromycin-antibody fragment complex is formed. The complex is then reverse transcribed, and a selection procedure is carried out. ssDNA is obtained by denaturation of the complementary RNA in an alkaline solution, and the target DNA sequence is amplified by polymerase chain reaction (PCR) following the selection procedure [31].

One drawback of this method is the lack of efficacy of the mRNA-protein complex formation. Nagumo et al. overcome this problem by unanticipated substitution mutations at the initiation codon of the antibodies. These base replacements disturbed the secondary structure of mRNA, which in turn resulted in more complex formation and enhanced protein expression [32].

3.2 Transgenic animals

Since the phage and other display methods have technical intricacy, other strategies have been applied to produce the next-generation mAbs, including transgenic animals. The human antibody genes consist of the reiterative and highly conserved groups of sequences are re-combined in B lymphocytes of mice to generate a highly diverse antibody library. This recombinant library subsequently can be applied to isolate against a certain antigenic epitope. Natural processes including DNA rearrangement and recombination, somatic hypermutation in hotspot regions, and clonal B cell selection occur since transgenes are under the regulation of the mammalian's immune response. Transferring recombinant genes into embryo pronuclei by microinjection technique employing retroviral plasmids or transposable elements is one approach of transgenic animal production [12]. Another method is sperm-mediated gene transfer (SMGT) and, subsequently, in *vitro* fertilization using Intracytoplasmic Sperm Injection (ICSI). Pigs and mice are preferred animals for generating a remarkable number of transgenic models using the ICSI technique (**Figure 1C**). The major challenge related to transgenic models is the cost and time needed to produce antibodies. Transgenic cattle also was designed for the high-level generation of bispecific scFv against melanoma, but problems to sustain stability and biosafety have yet to exist [33].

3.3 Single B-cell technique

The single B-cell technique is a unique approach that leverages the natural or activated human immune system to design *in situ* mAbs by immortalization of B lymphocytes with the Epstein-Barr virus. The essential benefit of this approach is the selection and production of natural antibodies of human origin with the native

VH-VL fusion, which cannot be produced in phage and transgenic model techniques. Various strategies exist, including fluorescent-activated cell sorting (FACS), micro-engraving, and fluorescent-activated droplet sorting (FADS) for the selection of single lymphocytes that produce target antibodies. FACS is a professional format of flow cytometry in which cells are stained with fluorescent-tagged molecules (antibodies and antigens). Tagged cells are further sorted into various subtypes according to their immunophenotyping features and migration toward a detector in fluid flow rate [34]. Compared to other screening approaches, FACS possesses the profit of being conceivable to richen a group of desirable single B lymphocytes more rapidly and completely. The selected group is further categorized relying on electrical charge (positive, negative, or neutral) to discriminate different phenotypes of a specific antibody (**Figure 1D**). Another technique for the selection of single cells in nano-amounts with the production ability of specific antibody, enzymes, or cytokines is FADS. FADS, like FACS, utilizes fluidic-based microchips to identify the existence of a particular antibody in the fluid flow. This technique can solve several problems related to FACS, including the potential to monitor produced antibodies that exist in the microfluidic system. Although single B-cell technology has been used successfully for mAb production against fungal and viral infections, this method has certain problems, including stability limitation for immortalized B lymphocytes, and cannot be utilized in all clinical situations [35].

3.4 Production of antibody and its fragment versions in heterologous systems

Different expression hosts have been employed to generate various biotherapeutics ranging from mAbs to their fragment derivatives. *E. coli* was the first system applied for expressing antibody fragments due to its high-production levels and affordable platform. Bacterial protein transport occurs *via* two most common routes, including the Secretion (Sec) route or the twin-arginine translocation pathway, depending on their signal peptide types. However, the main problems related to prokaryotic expression are low translocation efficiency into the extracellular space, intracytoplasmic misfolding of highly produced antibodies, endotoxin impurities, and the absence of glycan modifications. In the case of monoclonal antibodies, bacteria are not proper expression systems due to their incapability to glycosylate antibodies in the Fc portion. In contrast to full antibody molecules, fragment format of antibodies does not require to be glycosylated and, thus, can be produced at high amounts in bacterial expression systems, for example, Fab (over 4.0 g/L) and scFv (up to 3.5 g/L) using ESETEC bacterial secretion technique. However, protein folding and functional biotherapeutics production resulted in establishing mammalian cell lines as a preferred platform [36].

Above 60% of all biotherapeutic products, such as mAbs, are generated employing mammalian expression platforms. Post-translational modifications (PTMs) including glycosylation, appropriate folding, stability, and maintaining biological function occur in these expression systems in a similar procedure to humans. Because non-natural glycosylation pattern often causes several terminal glycan immunogenic epitopes, including N-glycolyl neuraminic acid (NGNA) or galactose-α-1, 3 galactose (α-Gal), which not existed in natural glycosylated proteins. These antigenic patterns trigger the human immune system, which can disrupt treatment efficiency through elevated elimination or, in more uncommon situations, lead to serious adverse effects [37]. On the other hand, inappropriate glycosylation impacts biological function

and stability of target protein. Therefore, glycosylation profiling of the mammalian recombinant expression systems requires to be evaluated to vouch their compatibility and lack of a-Gal and other immunogenic patterns with non-human nature. Besides, these engineered cell lines have higher productivity in bioreactors up to 10–15 g/L for mAbs and Fc-fusion protein expression [38]. In the case of fragment derivatives of antibodies, other producer hosts, including plants and insects, could benefit more than the mammalian cells platform due to their simpler manipulation and facility of employment for high-yield antibody production with high purity, functionality, and stability. The produced antibodies in herb can be transported outside of the cellular environment or remain in the endoplasmic reticulum (ER) to assist with the appropriate folding and prevention of misfolding, self-degradation, and proteolysis outside of the cell. However, drawbacks for antibody isolation and plant purification influence the final product's amounts. On the other hand, their glycosylation patterns may differ compared to humans and, in some cases, have low production amounts [39]. The baculovirus vector is the best platform for expression in insect cell systems. In contrast to prokaryotic expression systems, insect cells can perform desired posttranslational alterations at a higher extent, similar to mammalian systems. To overcome discrepancies in glycosylation patterns, engineered insect systems are used to produce the recombinant enzymes needed for producing glycosylated antibodies with similar patterns to those of humans. Since the baculovirus infection leads to lysis of insect cells, manipulated insect cells are used to produce the desired recombinant protein permanently [40].

4. Structure-based antibody engineering

Genetic and protein engineering techniques aim to enhance the bio-physicochemical characteristics of the desired antibodies for the highest efficiency. Technically, there are two approaches to attain this purpose. In rational design, structural data obtained from X-ray diffraction, NMR spectroscopy, and computational simulations were employed to manufacture a small collection of variants. In the second strategy or experimental method (directed evolution techniques), large libraries are constructed by applying phage, ribosome, or yeast display for the selection of the best antibodies in terms of their affinity, specificity, and functionality. The advantages of structural comprehension and rational design are multifold. In the humanization process, it assists in recognizing the crucial residues in the external regions of CDRs that must be conserved and residues inside of CDRs that need to be substituted. The technique identifies an amino acid that otherwise may be accounted as an unchangeable residue during the affinity maturation process. For solubility enhancement, alterations of the uncharged regions of the antibody superficial regions are needed [41]. Moreover, NMR, X-ray diffraction, and lately cryogenic electron microscopy (cryo-EM) have developed as supplementary methods to achieve conformational models of Ag-Ab complexes. If the empirical structural data do not exist, models generated by homology-based approaches are usually regarded as a reasonable replacement. Nevertheless, despite the apparent progress in the *in silico-based* approaches, the accuracy of forecasted antibody structure, especially in the case of CDR-H3, is insufficient, and the information on the Ag-Ab interface is also not completely accurate. Thus, homology is not an alternative to the experimental data, but as assistance that can improve computational design and evaluation of antibody mutant variants [42].

5. Humanization methods for monoclonal antibody production

All therapeutic antibodies produced in non-human species, such as rodents, chickens, and rabbits, against human targets need to be humanized. For instance, to decrease immunogenicity due to anti-mouse antibodies, the first effort for humanization of monoclonal antibodies produced in non-human species was generation of chimeric versions by fusing the variable regions of non-human mAbs with constant regions of human ones to produce mAbs with minimally 70% similarity to human origins. In the latter attempts, a CDR-grafting method was introduced by Winter and colleagues [43]. The strategy consists of the CDR fusion from a non-human (frequently mouse) donor antibody to the backbone of an antibody of human origin. In addition to grafting approach, other humanization techniques including resurfacing, super-humanization, and optimization of human string content have been established. These methods need the evaluation of residues to analyze the possible influence of each residue replacement on 3D conformation and biological activity of the molecule [44]. Approximately a few humanized antibody versions are commonly generated and screened for interaction affinity and biological function. If these antibody versions do not possess the bioactivity parameters, another round of development, alteration, and construction is performed to enhance interaction affinity. In the grafted CDR technique, selecting the CDR frontiers is optional and not restricted by the general concepts. Hence, some parameters are required to be taken into account. Initially, the CDR sequence ought to be small enough to minimize the number of non-human amino acids. Secondly, all amino acids within CDRs are required to interact directly with the target epitope. Besides, nearly 20% of amino acids are outside the CDRs. These residues are substantial for epitope interaction, similar to amino acids inside the CDRs, and in some instances, even more substantial thermodynamically. For shorter CDRs, more FR amino acids are usually needed, while fewer FR amino acids are required for longer CDRs in the back mutation technique [45].

The next step in the humanization process involves identifying human FR donors. In the constant FR method initially, a known antibody structure of human origin was used irrespective of its similarity to non-human antibodies. Besides, VH and VL donors were typically chosen from one molecule to optimize interactions. However, this approach often resulted in a significant or complete loss of affinity, prompting the adoption of a method known as "best fit." In this technique, the VH and VL human domains that are most similar to the non-human variant were chosen to generate humanized variants with greater affinity than antibodies achieved through the constant FR approach. Another approach to use human FRs as a model for humanization is generating consensus sequences. Human FRs can be selected from two sources including mature and germline. Mature sequences, formed during the immune response, contain somatic mutations and may be immunogenic. On the other hand, human germline sequences have lower immunogenicity and have been used as FR donors [46].

There are various methods for choosing a human germline for CDR grafting. One approach considers the general sequence similarity between the non-human variant and human germline in the variable sequences. Another common method is to focus on sequence similarity only within the FR sequences, without the CDRs consideration. The reason beyond this is that identical FRs generate a similar backbone for the CDRs and maintain 3D structure, but the CDRs sequence always stays unchanged (they are grafted). Another approach involves regarding sequence similarity inside the CDR sequences based on the standard structures mainly identified by the CDR

domain. This approach is named super-humanization. Generally, one germline is chosen for the VH and another for the VL domain. However, when FR donors are chosen independently, a combinatorial method is employed, and sequences fused from various germlines are obtained. The approach possesses the apparent benefit of more pliability by selecting human germlines with more similarity scores. Sequences originating from multiple FRs may represent inconsistency when consisting of various germlines. Deep-learning (DL) and machine-learning (ML)-based computer software such as Rosetta Antibody modeling can predict the VH-VL orientation and may assist in identifying the optimum couple of germlines [47].

6. Back mutation technique

CDR grafting may decrease target recognition despite conserving heavy-light chain-interacted amino acids. This issue often arises from reciprocal discordance between non-human CDRs and human FRs. Hence, each CDR grafting approach should consider a step to recognize critical FR residues for preserving the 3D structure of CDR. In cases where an amino acid from a non-human source in a crucial position cannot be maintained due to dissimilarity with human sequences, a back mutation is necessary. This involves mutating an amino acid in the human framework region to the residue present in the non-human parental antibody. Although this alteration decreases the antibody's humanness score, the substitution will enhance the binding affinity [48]. Residues directly interacting with the CDRs, which can potentially affect the CDR 3D structure, are responsible for organizing the Vernier zone. Thus, back mutation of most of Vernier zone amino acids is a usual practice only to decrease the feasibility of an intervening influence of human amino acids on interaction ability. However, this event will unavoidably introduce some "non-human" amino acids to the humanized molecule. Therefore, in the process of humanization, a precise evaluation of the importance of each Vernier zone position in relation to specific CDRs and Ag-Ab interactions is fundamental [49]. In addition to the constraints of antibody modeling, the lack of complete data about the CDR engagement in epitope interaction often leads to additional back mutations during the humanization process. To avoid these effects, each potential important residue should be evaluated for back mutation, and only the amino acids affecting the interaction should be mutated in the designed variants. Back mutations are also used to restore interaction affinity and improve the translation of humanized antibodies [42].

7. Deimmunization approaches

Although some amino acids in FRs may need back mutation, some residues inside CDRs that do not participate in the antigen bindings or do not affect the CDR 3D structure may be changed to human germline sequences. The reason beyond the inclusion of human germline amino acids into the CDRs is that they likely represent the sole regions in humanized and completely human variants. By substituting only 1 or 2 residues inside each T-cell epitope, the immunological responses of the antibodies could be decreased while preserving their binding affinities. The technique, known as deimmunization, can be considered supplementary to the back mutation technique [50]. EpiSweep is a structure-directed deimmunization approach and was introduced by Parker et al. The technique recognizes a series of substitutions in possible antigenic

sequences, generating optimum exchange between structure and immunogenic potential. It involves dynamic energy calculations and epitope prediction tools [51].

8. Resurfacing and super-humanization techniques

Another method of decreasing the immunogenic potential of the humanized variant is to substitute just the superficial amino acids in the non-human version with human ones. In contrast to CDR grafting, resurfacing preserves the hidden amino acids of the non-human version. The technique envisaged removes probable immunogenic epitopes and reduces the disturbance of amino acids in the epitope-paratope recognition site. A comprehensive evaluation of molecular sequences was conducted to identify the important amino acid distribution that contributed to solvent accessibility in mouse and human variants. Similarity analysis revealed that sequences in corresponding positions on the superficial regions of variable domains in both organisms are preserved with 98% accuracy through species. Consequently, only minimal alterations in residues are needed to change a mouse Fv surface template to that of a human Fv [52].

Contrary to CDR grafting, affinity is preserved in the resurfacing process since it incorporates a limited number of substitutions located on the antibody's superficial regions and usually does not alter variable domain conformation. However, the residues that existed in the variable regions stay fundamentally non-human and can generate possible MHC II epitopes irrespective of exposing their superficial regions. FRs with human germline gene repertoire origin may be chosen for CDR grafting based on the greatest sequence identity inside FRs or CDRs. In the latter technique, the FR similarity is not crucial. The approach is known as super-humanization [47]. It is important to declare that super-humanization has also been employed in different contexts, specifically in instances of humanized variants possessing somatic mutations in FRs to enhance their humanness, as calculated by the germinality score. Clearly, in those situations, no CDR grafting is required and super-humanization ordinarily demonstrates a greater human context of the designed molecule [53].

9. Humanness optimization

The humanness of the variant can be analyzed by each index that is capable of distinguishing non-human from human amino acid residues. The human string content (HSC) value assesses the ratio of human germline amino acids inside a specific molecule. The score is computed for a protein in the interested location by calculating the amino acids that match their counterparts in the most closely related peptide from a human germline source. This score is utilized for the humanized version by magnifying the value instead of employing an overall similarity calculation to produce several different humanized versions. The designed variable domains vary essentially from antibodies generated by the CDR-grafted technique in terms of immunogenicity due to being more humanness since they originated from some distinct human germline antibodies [54].

Since humanness optimization uses positional sequence from several germlines, prudence of 3D-conformational structure is reasonable to prevent clashes. Bioinformatics screening according to analogous contact environment (ACE) algorithm for filtering reciprocal consistency of various sequences analyzes motif regions

of residues for priority in the antibody dataset. The motif priority value in the target location calculates the similarity index according to dissimilarity and identity weighting for the most identical region in the dataset. Means of entire amino acids generate the overall motif priority for the antibody. A higher priority score demonstrates that homologous motif environments are gathered in the dataset, proposing that the evaluated structure is probably more favorable [55].

10. Switching of lambda chain to kappa

Following the humanness process, the light chain isoform of the template molecule is commonly not altered; for instance, in the non-human molecule, the FR for a humanized version is chosen from the lambda of human origin. However, the VL-type switching can have distinct advantages in several cases. For example, generating bispecific molecules with isotype of different light chains leads to one-step purification, which could be easily optimized. This information demonstrates that lambda and kappa isotypes are exchanged without disruption of the biological activities of the molecule. The approach is used in the humanness process or can cause benefits during the thermodynamic characteristic optimization of bio-therapeutics [56]. One favored instance of lambda to kappa isotype switching for enhancing the biophysical features of antibodies was generated in IGHV1-69*01 germline scFv. The obtained scFv demonstrated improved thermostability and production amounts while preserving its binding affinity to the specific antigen. These data indicate that λ and κ chains switching not only does not compromise the biological activities of the antibody but also can be employed in antibody humanization or improve the biophysical features of therapeutic mAbs [57].

11. Affinity maturation engineering

Both human and non-human natural antibodies usually do not have the desired binding affinities needed for their therapeutic utilities. Therefore, enhancing the binding affinity is a significant and approximately unavoidable stage for producing efficient mAbs due to its association with the amounts required for effective medication [58]. Many *in vitro/vivo* techniques are available for affinity maturation strategies. Commonly, rational engineering methods are only partially suitable for CDR mutations to increase their interaction affinity since they cannot approximate the molecular dynamic structure in the Ag-Ab complex [59]. Thus, a recently established web-based software known as mCSM-AB (http://biosig.unimelb.edu.au/mcsm_ab/) is available that utilizes free energy alteration during variation and appraises the interaction fluctuation. In this software, a negative mark indicates reduced interaction ability results from chosen variation, and a positive mark demonstrates higher interaction power. This software analyzes the impact of several mutations in one mAb simultaneously. It is important to note that desired mutations for improving binding affinity should not reduce other biophysical properties of an antibody, such as solubility and stability [60]. Novel strategies by applying NMR relaxation scattering and mass spectrometry investigations are available to specify sequence locations to maximize the interaction. If empirical data are unavailable, *in silico* modeling may facilitate obtaining optimized affinity [61]. Many investigations demonstrate that mutation locations are required to be at the circumference of the Ag-Ab complex, and residue replacements ought to be ones that commonly change following affinity

maturation within the body. Therefore, affinity-improving substitutions desire to accumulate surround locations where somatic mutation happens naturally [62].

On the other hand, integrating the computational analysis and thermodynamic calculations provided an efficient approach to increase the affinity. Besides, by choosing the locations for mutations to increase binding in the paratope amino acids or Vernier zone-surrounded amino acids, one will be able to consider locations in the center of the VH regions or even at the junction site of variable and constant regions [61, 63].

12. Specificity engineering

Specificity is a key antibody feature and a crucial factor affecting the efficiency of immunoglobulin drugs. It plays a critical role in the success of antibodies as therapeutic candidates, since non-specific bindings or cross-reactivity can lead to off-target interactions and rapid antibody elimination within the body. In experimental conditions, self-interactions cause immunoglobulin aggregation, low solubility, and abnormally greater viscosity [64].

Similar employed combinational procedures to increase binding affinity can also be utilized to alter antibodies cross-reactivity. The use of computational engineering methods and the availability of empirical structural data are a major factor in specific design of antibody. Recently, a novel approach to engineer antibody with high affinity has been developed without need to previous information of the binding sites at the interface between antibody and antigens. This approach utilizes a highly diverse library of phage displayed Fab variants containing entire feasible single-base substitutions in the different CDRs [65]. The lack of Ag-Ab binding data in this strategy were gratified by constructing a library with higher diversity. The flexibility of the antigen interaction site, which allows the identification of configurationally irrelevant epitopes by a single antibody, is another strategy for multi-specificity. The approach was employed in a step-by-step manipulation method to produce bispecific antibody *de novo*, known as a two-in-one molecule with dual-action Fab (DAF).

12.1 Designing by rational methods

The essential parameters influencing antibody production are solubility, stability, and agglomeration. These problems can exist during the developmental process and can cause a decline in antigen-binding affinity, immunological stimulations, and waste of resources. Both sequence and conformation are key factors involved in agglomeration and stability characteristics of an antibody. Handling these features with rational design prior to experimental and *in vivo* investigations is beneficial [66]. Rational design strategies aim to identify challenging antibody domains related to direct sequence or conformation. Thus, integrating rational design techniques and experimental and *in vivo* methods concerning intrinsic parameters, including physicochemical properties, pH, ionic strength, temperature, and replacing agglomeration-susceptible amino acids increase the selection of highly soluble and stable molecules in the initial steps [67].

12.2 Protein engineering techniques for improving solubility, stability, and specificity

Initial investigations demonstrated that antibody agglomeration and thermodynamic properties are measurable, and antibody design can be carried out according

to favored features. Antibody manipulation techniques can commonly increase interaction/specificity, improve solubility and stability, and inhibit agglomeration without interaction/specificity alteration [68]. For instance, a new technique known as stress selection based on phage display was applied to explore nanobodies with higher stability and improve thermodynamic properties and solubility. In this technique, random mutagenesis PCR was used to generate highly diverse libraries and then expose them to different stress situations, including incubation at 50–80°C or acidic conditions during various periods and incubation with different proteases. The obtained data identified useful variations (both on CDRs and FR amino acids) that were the same under these stress conditions. While a majority of these alterations were found to be in CDR residues, it was noted that interaction affinity was only moderately affected by around half of the mutants. Before incorporating alterations to reduce agglomeration and improve protein stability, some fundamental issues should be considered, including the following: (1) avoid mutating the CDR portions despite high predicted scores, due to their key role for epitope recognition, and altering them may affect affinity and specificity; (2) first, exposed hydrophobic residues that promote aggregation should be replaced with hydrophilic ones; and (3) compare engineered mutants with natural variants since changing an amino acid to its naturally conserved residue potentially enhances biochemicophysical features [69]. The abYsis databank is an online software incorporating sequence information from the European Molecular Biology Laboratory/European Nucleotide Archive (EMBL-ENA) and structural information from the Protein Data Bank (PDB). This databank is employed to pinpoint position-specific residues of the natural variants in various species [70].

Forecasting of potential aggregation-prone regions (APRs) of mAbs is carried out by aggregation/solubility prediction software with the highest accuracy. Based on their sequences, these tools analyze the amyloid formation and agglomeration tendency/APRs of mAbs [71]. Tango, the initial web-based software for forecasting agglomeration (http://tango.switchlab.org/), evaluates the β-sheet amino acid agglomeration of a mAb by assigning plausibility values to beta-sheet and turn, alpha-helix, and beta- and alpha-amyloid for each residue incorporating particular external properties (temperature, pH, and ionic concentration). The tool supposes antibody sequences with a high agglomeration tendency if they contain at least five sequential amino acids with a plausibility of adopting the β-amyloid formation exceeding 5% per amino acid. Research has shown that the software achieves an 87% precision rate, accurately predicting 155 out of 179 proteins, with 21 false positives and 3 false negatives [72].

Aggrescan3D (A3D) (http://biocomp.chem.uw.edu.pl/A3D/) is an upgraded copy of Aggrescan, addressing the constraints of sequence-driven assessments. This tool enables the evaluation of how individual mutations influence the propensity of proteins to aggregate [73]. These approaches can be used alone or in conjunction to forecast aggregation/solubility indices. Integration of these different strategies can improve the accuracy of mutational investigations. These methods assess mutant versions and natural antibody features to calculate changes [74].

A stable antibody can be determined by analyzing the alterations in free energy caused by residue variations, with lower free energy scores indicating greater stability. Different techniques, such as biochemical, mathematical, experimental, or artificial intelligence-based computational methods, have been applied to forecast antibody stability [75]. For example, ProMaya (http://bental.tau.ac.il/ ProMaya/) utilizes a collaborative filtering [CF] approach and accidental forest regression to measure the

free energy alteration caused by amino acid variations. The software also proposes that determining the difference in free energy amounts at a specific site improves the free energy changes forecasting elsewhere [68]. Another software named SDM (http://marid.bioc.cam.ac.uk/sdm2) analyzes the natural and mutant antibody differences by utilizing a structurally limited environment-specific substitution table (ESST). This method considers factors such as amino acid compacting density and the ESST to determine the impact of residue alterations on stability. The tool predicts stability changes as decreased, increased, or unchanged [76]. I-Mutant (https://folding.biofold.org/cgi-bin/i-mutant2.0.cgi) forecasts alterations in antibody stability according to the support vector algorithm and permits applicants to employ sequences or structure to forecast with an accuracy of 77–80% for the databank retrieved from ProTerm [77]. Cupsat (https://cupsat.brenda-enzymes.org/) utilizes atom potential and turns hermitage dispensation data of residues to determine antibody stability based on the thermodynamic free energy alteration due to mutagenesis with 80% accuracy. The software evaluates the antibody configuration and provides data regarding mutagenesis position and solvent accessibility, and whether the altered residue has proper turn hermitage [78].

Certain PTMs carried out on a specific antibody have essential significance for their therapeutic function. Given the temporary and diverse characteristics of PTMs, the high specificity and binding interaction of PTM-specific antibodies are crucial for their accurate and consistent discrimination. Previous works established a new technique utilizing a surface display approach in yeast systems to calculate the specificity of PTM-specific antibodies. This method allows us to evaluate their interaction and detect any cross-reactivity with non-target. The study demonstrated that site-directed mutagenesis is a crucial method for achieving PTM-specific antibodies with great specific interaction. It emphasized the significance of the specificity evaluation in the protein engineering technique [79].

13. Gene immunoediting techniques for engineering next-generation antibody libraries

The realm of directed mutagenesis and antibody manipulation techniques also benefit from highly automated gene editing. Lately, great-proficient homology-directed repair or HDR (above 30%) was developed employing random single-stranded oligonucleotide (ssODN) donors to design specific mutation repertoire. Biogenesis of ssODNs is commonly constrained to an utmost length of two hundred bases and inappropriate for the incorporation of VH repertoires (above four hundred nucleotides) [80]. Plug-and-(dis)play (PnP) hybridomas technology has been developed recently. In this platform, the hybridomas genome is edited by HDR using CRISPR-Cas9 for the production of the designed target molecule. For this aim, an accidentally diverse repertoire was designed *via* error-prone (EP) mutagenesis on the heavy part and subsequently was inserted within vector and transfected in PnP cells. Analysis of this combined VH repertoire leads to selecting a series of specific antigen-binding mutants. In the next step, an enhanced Cas9 HDR method was applied to generate affinity maturation of the target antibody. Finally, enhanced variants were successfully selected, exhibiting affinities against the target antigen in the low picomolar range. Profound sequencing of these libraries resulted in a more complete examination of library versatility, choosing and richness, and unraveling mutational priorities, which all proposed that PnP analysis was a rigorous method for designing antibodies (**Figure 2**) [81]. Expanding libraries by error-prone PCR to generate diverse mutations in the light chain, variable domain shuffling, and

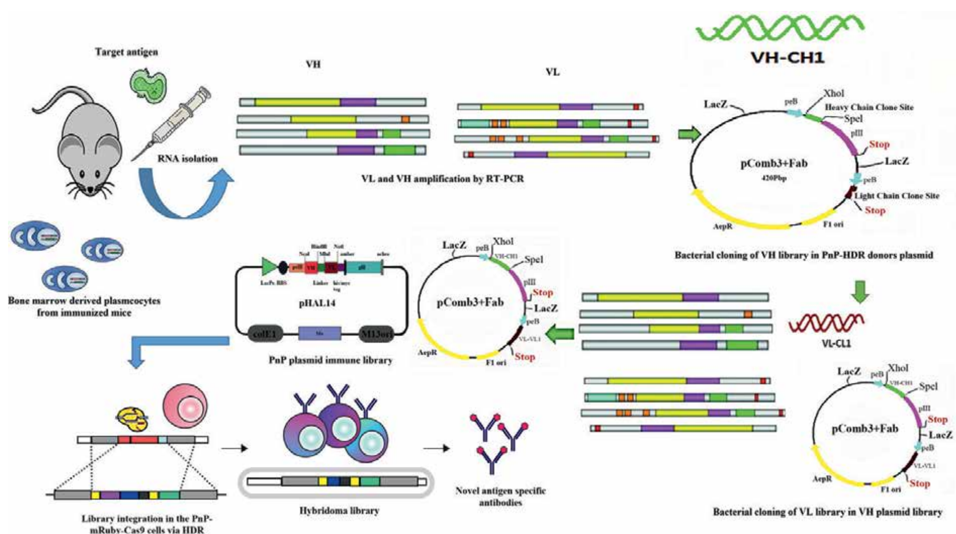

Figure 2.
Improved Cas9-forced HDR by generating a self-linearized donor plasmid. Picture illustrates the workflow to manufacture and display an immune library from bone marrow plasmocytes of an epitope-immunized mouse. The PnP workflow to reprogram mRuby hybridomas to produce a chosen antibody after integration of a recombinant synthetic antibody in the V_H locus. This optimized workflow of the PnP-mRuby cells permanently producing Cas9 is utilized with an HDR donor plasmid carrying a recognition site for the same Cas9 gRNA that is applied to cut mRuby (protospacer adjacent motif is indicated in red). After entrance of the plasmid and the gRNA complex into the nucleus, Cas9, which is also entered to the nucleus because of its nuclear localization signal, is recruited to both trigger a DSB in the genomic mRuby coding sequence and linearize the plasmid, rendering it more prone to integration by HDR.

merging the useful mutagenesis of various variants can be favorably incorporated for PnP analysis. In comparison with display systems, PnP technology has the essential benefits of choosing antibodies in the natural mammalian system and can assist in the selection of specific mAbs with the desirable therapeutic effects. PnP hybridomas technology has the capability to integrate libraries into particular genome locations of mammalian cells with higher HDR efficiencies. This method uses single-stranded oligonucleotides (ssODNs) as the donor template instead of double-stranded DNA, intensively enhances HDR integration proficiencies, and also diminishes off-target integration events [82].

14. Novel approaches in Fc-engineering for enhancing effector functions

The effector activities of naturally produced antibodies by immune systems or synthetic form for therapeutic application depend on the binding of the Fc part to FcγRs and activation complement system *via* complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP). Optimization of this binding potential has become a hopeful strategy to improve the efficacy of therapeutic antibodies in treating tumors and autoimmunity [83]. Strategies including *in situ*-specific mutations and glycosylation manipulation to improve FcγR interaction, Fc multimerization, antibody hexamerization to accelerate C1q interaction *via* "HexaBody" technology, and cross-Isotype antibodies have been developed to improve effector mAbs function as listed in **Table 1** [84]. Despite the advancements brought by the hybridoma technique

Fc-manipulated mAb	Target	Isotype	Chimeric/ Human(ized)	Fc manipulating Approach	Enhanced effector function	Additional mAb engineering	Clinical stage; NCT of recruiting clinical trials	Major indication(s)
Obinutuzumab (GA101;Gazyva)	CD20, type II	IgG2	Humanized	Afucosylation	ADCC	Modified elbow hinge	FDA-approved	FL and CLL
Ublituximab (LFB-R603, EMAB-6)	CD20, type I	IgG1	Chimeric	Low fucose	ADCC		Phase 2/3	CLL and B-NHL
Ocaratuzumab (AME-133v, LY2469298)	CD20, type I	IgG1	Humanized	Mutations P247I/A339Q	ADCC	Antigen binding affinity optimized	Discontinued	
PRO131921 (RhuMAb; v114)	CD20, type I	IgG1	Humanized	Mutations (na)	ADCC and CDC		Discontinued	
Ocrelizumab	CD20	IgG1	Humanized	Mutations (na)	ADCC		Discontinued in hematology, approved for MS	MS
CD20 double engineered	CD20	IgG1		Afucosylation + mutations S267E/ H268F/S324T/G236A/ I332E	ADCC and CDC		Preclinical	
	CD20	IgG1/ IgG3		Afucosylation + mixed IgG1/IgG3 isotype	ADCC and CDC		Preclinical	
BI 836826	CD37	IgG1	Chimeric	Mutations S239D/I332E	ADCC		Discontinued	
DuoHexaBody-CD37 (GEN3009)	CD37	IgG1	Human	Mutation E430G (HexaBody)	CDC	Dual-epitope targeting	Phase 1	B-NHL
Ianalumab (VAY736; B-1239)	BAFF-R	IgG1	Human	Afucosylation	ADCC		Phase 1	CLL
Inebilizumab (MEDI-551)	CD19	IgG1κ	Humanized	Afucosylation	ADCC		Phase 1/2	B-cell malignancies

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Fc-manipulated mAb	Target	Isotype	Chimeric/ Human(ized)	Fc manipulating Approach	Enhanced effector function	Additional mAb engineering	Clinical stage; NCT of recruiting clinical trials	Major indication(s)
MDX-1342	CD19		Human	Afucosylation	ADCC and ADCP		Phase 1 halted	
Tafasitamab (MOR208, XmAb5574)	CD19	IgG1	Humanized	Mutations S239D/I332E	ADCC and ADCP	Antigen binding affinity optimized	Priority review granted by FDA	CLL and DLBCL
HexaBody-CD38 (GEN3014)	CD38	IgG1	Human	Mutation E430G (HexaBody)	CDC		Preclinical	B-NHL and MM
Anti-CD38 SIFbody	CD38			Fc multimerization	ADCC and CDC		Preclinical	MM
XmAb5592	HM1.24	IgG1	Humanized	Mutations S239D/I332E				MM
	ICAM-1	IgG1	Human	Mutations S239D/I332E	ADCC and ADCP		Preclinical	MM
SEA-BCMA	BCMA	IgG1	Humanized	Afucosylation	ADCC and ADCP			

Table 1.
Examples of human Fc mutations or modifications for alteration functional effector activities of mAbs.

in the realm of ADCs, the majority of mAbs currently approved for clinical applications are produced using mammalian host platforms. These platforms enable the production of larger quantities and superior-quality antibodies by conserving correct PTMs. Novel techniques for manipulating sequences at the Fc domain are available including *in situ*-specific mutations, which can be applied directly after obtaining VH and VL sequences (for conformational-based sequence design) or by creating highly diverse libraries to identify the most effective Fc mutants (experimental-based sequence design) [85].

15. Epitope mapping techniques for production of new classes of monoclonal antibodies

The procedure of identifying the sequence identity and site of a single specific epitope is called epitope mapping. There are two categories of epitope mapping procedures including sequence and functional-based type. Sequence strategies identify biological groups near the antibody, while functional-based approaches investigate the specific functions of each antigenic component in identification and their respective energetic involvement. X-ray diffraction study of Ag-Ab interactions is the most precise approach for mapping conformational epitopes [86]. This technique ensures accurate discrimination of linear and also conformational epitopes and provides data on the binding affinity. Nonetheless, X-ray diffraction is restricted by the crystals resolution and the electron bulk of the molecule [87]. Lately designed user-friendly accessible software, FTProd, serves as an *in silico* alternative to expensive and time-wasting X-ray diffraction [88]. Conventional X-ray diffraction can be substituted by NMR. The method supplies information regarding the sequence, conformation, and free energy of the Ag-Ab interaction and is achieved in a solvent without crystal formation requirement. Nonetheless, the technique is applicable only to low molecular weight (lower than 25 kDa). Saturation transfer distinction-NMR and antibody inhibition of hydrogen-deuterium exchange in the antigen are techniques suitable for mapping epitope regions with moderate resolution [89]. Electron microscopy (EM) is another technique for epitope mapping; nevertheless, the technique determines molecular structure with low quality and is applicable to larger antigens (for instance, entire viral particles). This technique cannot identify interacted amino acids, but it is suitable for validating the surface exposed epitope. Cryo-EM is another technique that enables the evaluation of Ag-Ab interactions following quick freezing in physiological buffers without the requirement for staining and fixation procedures [90].

Function-based epitope mapping techniques can be classified into four major clusters: competitive, antigen digestion, modification techniques, and techniques based on peptide library construction or synthetic epitopes. Competition techniques have a limited mapping quality. They are commonly used to assess whether two different mAbs are able to interact with an antigen simultaneously or compete for a similar epitope [91]. Most functional techniques are derived from the capability to discriminate the Ag-Ab interactions by manufactured epitopes or recombinant mutated versions of epitopes expressed utilizing display technology. In the interaction assay, epitopes are fixed on a solid surface, and ELISA, Western blot, and dot blot can detect the binding of antibodies. This technique does not need costly instruments and can measure the binding affinity toward a particular antigen. The epitopes are constructed on pins (PEPSCAN), cellulose filter surfaces (spotting technique), or epitope microchips [92].

Generating different mutations is a fast epitope mapping technique that depends on substituting single key amino acids that form immunogenic peptides and induce a lack of antibody interaction. These key amino acids are located on hot spots and constitute only a small portion of the Ag-Ab binding surface. Either accidental or *in situ*-specific mutagenesis can generate the protein library. Combining the mutagenesis approach with display technologies facilitates the analysis of highly diverse epitope variants [93]. Another universal technique is the saturation mutations substitute residue with all naturally existing amino acids at a specific position. Nonetheless, in certain instances, the lack of immunoreactivity because of the disruption of antigenic conformations can complicate the interpretation of the findings. Shotgun mutagenesis, another technique in mutagenesis, facilitates the discrimination of continuous and discontinuous epitopes. This technique relies on extensive mutations, where individual clones carry a specific residue change, followed by a simple cellular screening for monoclonal antibody reactivity toward native proteins [94]. Display techniques create a robust method for epitope mapping. The technologies are high-throughput and affordable. Building peptide libraries on phage surfaces illustrates a favored way of generating antigenic fragments for antibody binding screening [95].

16. Novel techniques for designing of the next generation of CAR-T cells and CAR-NK cells in the field of personalized medicine

Introducing therapeutic products of chimeric antigen receptor (CAR)-T cells for human-personalized medicine applications has created a breakthrough in curing diseases in oncology and hematology. Numerous human trials are carried out on next-generation CARs, which include a CD3z signaling domain, a surface epitope-recognition domain derived from a mAb, and one costimulatory element. Advancements in molecular tools have simplified designing the next generation of CAR-T cells with diverse biological functions, including extra costimulatory components, immune-checkpoint regulation, cytokine production, safety switches, or inactivation of therapy-intervening molecules (**Figure 3**) [96]. The inherent capability of natural killer (NK) cells to lysis cancerous cells, along with the specific and high cytotoxicity provided by transgenic CAR, has resulted in a technically feasible, efficient, and safe treatment option for different tumor types and antigenic targets. The intracellular domain of CAR-NK cells by recognizing its antigenic target sends a triggering signal, resulting in the production of cytokines and the destruction of the tumor cell [97]. Nowadays, there are four distinguishing methods tested in clinics to produce new versions of CAR-T/NK cells. Their aim is to improve treatment efficacy through the modulation of immune checkpoint pathways or cytokine secretion. Safety-switch tools can be used to manage unfavorable events such as cytokine storms by inactivating CAR-T cells with external mechanisms. Trials are being conducted to assess engineered CAR-T/NK cells for allogeneic application or to treat hematological and solid tumors [98].

16.1 Armored CAR-T cells: Checkpoint of immunological regulation

CAR-T cells aiming at checkpoint immunological regulation strive to overcome tumor microenvironment (TME) inhibition. All the clinical trials assessing the feasibility of this procedure depend explicitly on disrupting the PD-1 pathway. Interestingly, personalized medicine studies show distinctive approaches to

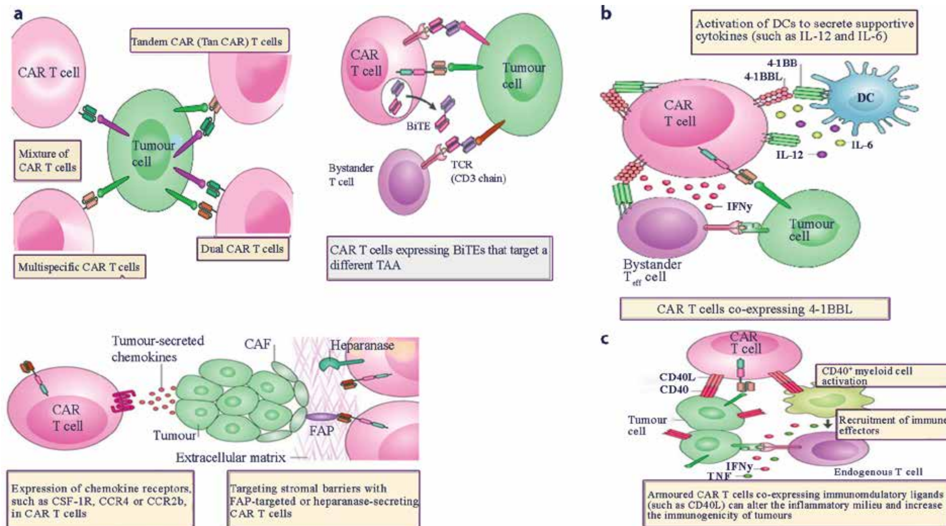

Figure 3.
Novel techniques for construction of next generation of CAR-T cells. a. In-site programmable CAR-T cells, b. TRUCKs—cytokine-producing CAR-T cells, c. Armored CAR-T cells. For details please refer to the text.

dysregulate PD-1 signaling. In NCT03258047 trial, the investigators utilized an innovative method in which the intracellular CD28 activating domain was connected to the extracellular PD-1. Therefore, PD-L1 binding to PD-1 was converted into a triggering signal, and consequently, more powerful antitumor properties occurred. Disruption of PD-1 in TME can also be overwhelmed by CRISPR/Cas9 engineered a novel version of anti-CD19 CAR-T cells to boost its anticancer activity. According to another study, PD-1 downregulation in anti-prostate cancer stem cell antigen CAR-T cells using a Cas9/RNP gene editing system significantly enhanced T cell immunity *in vivo* (**Figure 3c**) [99].

16.2 TRUCKs: Cytokine-producing CAR-T cells

TRUCKs (T cells redirected for universal cytokine-mediated destruction) are the next generation of modified CAR-T cells to produce specific cytokines, increase their anticancer effectiveness, promote longevity, and influence TME nature (**Figure 3b**). Clinical trials have investigated CAR-T cells expressing interleukin-7 and CCL19 (Chemokine (C-C Motif) Ligand 19). IL-7 supports T-cell survival and growth, while CCL19 attracts and recruits dendritic cells and T cells [100].

16.3 Switchable CAR-T cells

Switchable CAR-T cells have been designed to address the predominant toxic side effects contributed to traditional CAR-T therapies, such as neurotoxicity and cytokine storm syndrome. These innovative cells can be deactivated using safety switches that trigger apoptosis, ADCC, or CDC in response to a specific external stimulus. By incorporating transgenes that express efficiently targetable surface domains, switchable CAR-T cells can be precisely controlled and eliminated if necessary. One approach currently being evaluated in clinical trials involves introducing the truncated version

of the surface epidermal growth factor receptor (EGFRt) into CAR-T cells as a safety measure. The monoclonal antibody cetuximab targets EGFRt, enabling the removal of T-cells through ADCC or CDC mechanisms [101].

16.4 Global CAR-T cells and fratricide-resistant CAR-T cells

Genetic engineering achievements have facilitated the construction of various CAR-T cells with allogeneic sources. Extracting MHC and TCR molecules from donor-originated cells is essential to generate a universal CAR-T. This is made possible by tools like CRISPR/Cas9, enabling gene knockout. Progress in CRISPR/Cas9 technique in T-cell editing was highlighted in the first human trial NCT03399448. The investigation demonstrated the feasibility of multiplex CRISPR-Cas9 genes engineering in clinical conditions; it also showcased the tool's potential in creating fratricide-resistant CAR-T cells by removing T-cell-specific antigens. This approach empowers CAR-T cells to target a wide range of T-cell cancers [102]. Various studies have leveraged CRISPR techniques to develop allogeneic universal anti-CD19 CAR-T cells by knocking out multiple genes, including MHC, TRAC, and β2M. Underway clinical trials indicate that incorporating a suicide gene in the CAR structure can help avoid graft-versus-host disease (GvHD) post-allogeneic CAR-T cell infusion [103].

16.5 *In situ* programmable CAR-T cells

Conventional adaptive (*ex vivo*) T-cell-based therapeutics are an essential platform where a patient's T-cells are extracted, manipulated genetically outside the body, and then reinfused into the same individual based on personalized medicine treatment to combat tumors. Two T cell-based therapeutics targeting different cancers through CAR are already available in the USA. However, the high cost of $373,000 *for Yescarta and* $475,000 for Kymriah annually makes these therapies unaffordable for many patients [104]. *In situ* programmable T-cell modalities can create tumor-specific therapeutic products *de novo*, which actively migrate to the target site, proliferate, and eliminate cancer cells consecutively (**Figure 3a**). The *in situ* programmable CAR-T cell in the form of the nanoparticle can be easily mass-produced in a stable form, distributed conveniently, prescribed inexpensively, and given to large numbers of patients in outpatient conditions. Besides, unlike traditional adoptive CAR-T cell therapies that require rigorous chemotherapy preconditioning to deplete endogenous lymphocytes, this approach eliminates the need for preconditioning the recipient, avoiding unwanted immunological response challenges [105]. In the instance of *in situ* programmable CAR-T cells, preparative procedures are optional as transgene-carrying nanoparticles directly program existing T cell subsets in circulation. This reprogramming occurs without T cells leaving their natural environment or being exposed to abnormally high levels of cytokines. Unlike the traditional *in vitro* propagation method that can lead to functional depletion of cells before reinfusion, *in situ* programmed CAR-T cells are promptly deployed to target and eradicate tumor cells. This approach allows clinicians to initiate antitumor immunological stimulations immediately following diagnosis, reducing technological demands and expenses. This could prove crucial for individuals with quickly progressing diseases [106]. **Figure 4** illustrates the main advantages of *in situ* CAR-T generation.

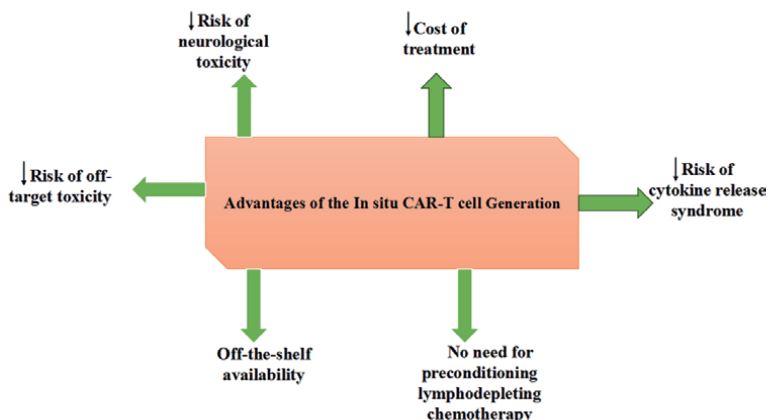

Figure 4.
The prominent advantages of the in situ *CAR-T generation.*

16.6 CRISPR-Cas9 and siRNA/shRNA (small interfering RNA)/short hairpin RNA) technologies to knockout or knockdown CAR NK cells

When NK cells are engineered to express a CAR, they maintain their innate capability to recognize tumor cells through their native receptors. They offer a more favorable safety profile compared to CAR-T cells counterparts. Despite CAR-NK cells' advantages over traditional CAR-T cells, their clinical use is restricted due to reduced persistence potential and proliferation *in vivo*, as well as manufacturing complexities. However, next-generation CAR-T cell engineering advancements can potentially harness and generalize all the benefits for CAR-NK cells [107]. The pros and cons of CAR-T and CAR-NK cells are outlined in **Table 2**.

Immune cell	Advantages	Disadvantages
NK cell	Multiple innate activating receptors that can mediate killing No need for previous antigen priming Can harness KIR-ligand mismatch and "missing self" to reduce risk of relapse Lack of cytokine release syndrome (CRS), and neurotoxicity Rapid tumor killing Multiple mechanisms of cytotoxicity Lack of graft-versus-host disease (*GvHD*)	Low persistence in the absence of cytokine Numerically few necessitating *ex vivo* expansion Suboptimal trafficking and penetration into solid tumors
T cells	• Links innate and adaptive immune systems • MHC independent TCR • Cross-present antigens to ab T cells • Better expansion and persistence	• Can have immunosuppressive properties • Numerically few necessitating *ex vivo* expansion • Higher cost and preparation time • Toxicity (CRS, neurotoxicity)

Table 2.
Advantages and disadvantages of T and NK cells as platforms for CAR engineering.

To boost the efficiency and function of NK cells for adoptive transfer therapy, CRISPR-Cas9 technology has provided good conditions by enhancing their antitumor effectiveness while modulating their exhaustion and persistence [108]. The potential of designing safer NK cell therapeutics products was demonstrated by knocking out MHC type I genes through β2M deletion using CRISPR-Cas9, followed by manipulating the NK cells to produce a single-chain MHC-E gene. These modified cells showed a similar phenotype to native NK cells, preventing incompatible T cell removal and NK cell fratricide, making them a valuable resource of non-MHC-compatible native NK as "off-the-shelf" immunological effector cells [109]. Similar to CRISPR-Cas9 gene engineering technology, gene silencing using siRNA or shRNA has been a well-developed procedure to reduce the expression of target genes, which has been employed to improve NK cell function in cell therapy. siRNA effectively boosts NK cell anticancer activity by downregulating NKG2A, an inhibitory receptor, thus prevailing NK cell exhaustion as genetically designed immunotherapeutic agents. In a study, lentiviral vectors carrying shRNA were used to target NKG2A downregulation on the NK cell surface, achieving 95% gene silencing [110]. Additionally, a combination of non-viral transfer of genetic materials generates a good potential, where Cas9 ribonucleoproteins (RNPs) with alpha polyglutamate polymer as carriers were delivered into NK cells *via* electroporation, improving transfection efficacy in CRISPR-Cas9 genetic engineering technology (**Table 3**) [114].

Car	Method	Targeted genes	Cancer	Reference
CD19 scFv/CD28/CD3 ζ	Cas9 RNP electroporation	TRAC B2M PD-1	B cell acute lymphoblastic leukemia	[103]
CD7 scFv/CD28/4-1BB//CD3	Cas9 RNP electroporation	CD7	T cell acute lymphoblastic leukemia	[111]
EBV-LMP2A CTL	Cas9 plasmid electroporation	PD-1	Epstein-Barr virus-associated gastric cancer	[112]
CD19 scFv/CD28/CD3 ζ	Cas9 RNP electroporation and transduced by AAV6 expressing CAR	TRAC exon 1	B cell lymphoma	[113]
CAR-NK cell	CAR.CD19-CD28-CD3ζ-iCasp9-IL15	CD19	B-lymphoid malignancies, ALL, CLL, NHL	NCT03056339
	RNP Electroporation	HPK1	Recurrent or refractory ALL and B-cell lymphoma T cell source autologous T cells	NCT04037566
	RNP Electroporation	TCRx, TCRB and PD-1	Multiple myeloma, melanoma, synovial sarcoma, myxoid/round cell liposarcoma	NCT03399448
	RNP Electroporation	B2M gene and TCR	B-cell leukemia and lymphoma	NCT03166878

Table 3.
Examples of genome-engineered CAR cells with CRISPR/Cas9.

17. Host engineering technologies for improving next-generation antibody production in the context of personalized medicine

Some methodologies for achieving improved expression proficiency and enhanced antibody quality originated from mammalian cell lineages were carried out by modifying the expression systems regarding cell growth manipulations, inhibition of cell death, and directing proper PTMs [115]. This aim is achieved extensively through various approaches such as modulating apoptosis, metabolic engineering, chaperone manipulation, cell growth engineering, and glycosylation modification. Strategies include delaying the onset of apoptosis through intermittent nutrient feeding or using alternative carbon sources such as galactose instead of glucose or adenosine utilization, upregulating of anti-apoptotic proteins such as Mcl-1, 30Kc6, Bcl-2, Bcl-w, Bcl-xL, Aven, E1B-19 K, and repressing pro-apoptotic proteins including Bax, Bok, Bak, and caspases. Additionally, inhibiting apoptosis *via* exosomes can enhance cell vitality, repress cell death, prolong cell culture lifespan, and improve the expression of interested antibodies. Suppression of mammalian cell cycle is another strategy that results in increased cell vitality, mass, and propagation in culture conditions by inducible production of cell-division controlling agents (p27 and p21cip1), prevention of cyclin-dependent kinase (CDK) or up-regulation of CDK inhibitor, and utilizing of rapamycin (through mammalian target of rapamycin (mTOR) pathway) [116]. Chaperones and foldases engineering, particularly upregulation of protein disulfide isomerase (PID) and BIP as a binding protein, impact expression amounts of antibodies *via* changes in the translational potential of the recombinant mAbs and folding-related pathway of secretory proteins, respectively [117]. Over-expression of X-box binding protein 1 (XBP1), involved in unfolded protein response, can also be applied for increasing recombinant mAbs expression merely when protein aggregation has outstripped the secretion potential of the expression system [118]. N-glycosylation situations on antibodies have been identified to have an essential effect on their functionality and are related to enhancing the efficiency and safety of the mAb. Any changes in the N-glycan type and their locations on antibody molecules can impact antibody-certain activities through modulation of ADCC and CDC of antibodies produced in CHO cells. Upregulation of N-acetyl glucosaminyl transferase III (GnTIII) enhances the bisecting GlcNAc levels to increase ADCC. Diminishing or removing the fucose levels on antibodies also significantly increases ADCC functionality by deleting the fucosyltransferase enzyme (FUT 8) from the CHO line, producing non-fucosylated antibodies [119].

Ammonia and lactic acid overproduction during engineered CHO growth *in vitro* is a usual process because of glutamine and glucose consumption and can cause negative impacts on the proliferating cells and secretion of recombinant mAbs. To prevent the accumulation of these toxic by-products, cells metabolically were engineered by upregulation of the glutamine synthetase (GS) gene to enable them to grow in a culture lacking glutamine, resulting in a remarkable reduction of ammonia production. Similarly, the upregulation of genes, including ornithine transcarbamylase or carbamoyl phosphate synthetase I (involved in the urea cycle), reduces ammonia generation within the culture medium [120]. Upregulation of pyruvate carboxylase or knocking down pyruvate dehydrogenase kinases/lactate dehydrogenase A (LDH-A) diminishes lactate accumulation. Simultaneous LDH-A knockdown and the GS enzyme expression in the mAb-expressing CHO remarkably diminish lactic acid and ammonia amounts in growth media [121, 122].

Cell growth in hypothermic conditions enhances recombinant protein levels in CHO cells since lower temperature detains cell growth, elongates cellular vitality, and enhances cellular size. Genetic manipulation techniques by upregulation of cold stress genes, including cold-inducible RNA-binding protein (CIRP), lead to an increase in the yields of recombinant antibody expression at cold conditions [80]. Cell manipulation techniques have also been employed to reduce main host cell proteins that may act as impurities and interfere with the final product purification in downstream steps or to identify a proper location in the host genome to integrate a transgene at that site. Cell engineering using enzymatic action of RNAs like ribozyme, especially microRNAs (miRNAs), presents a significant strategy to address industrial expressing host development issues. In the late years, the engineering of miRNA contents in CHO hosts has increased antibody amounts by enhancing cell growth and specific productivity, cell death resistance, and oxidative metabolism [80, 123].

Personalized medicine is a novel approach for treating and inhibiting diseases that focuses on individual genetic make-up, alongside environmental and lifestyle factors [124]. Personalized medicine seeks to categorize rather than personalize antibody therapies for specific patient subcategories with the relevant genetic backgrounds, moving away from the traditional “one-size-fits-all” drug approach. The field of pharmacogenomics, which examines how genetic differences impact antibody functions, is pivotal for identifying the most suitable medication and dosage for each patient. By anticipating or influencing treatment responses, personalized medicine aims to enhance antibody effectiveness and safety based on the patient’s genetic makeup, ultimately maximizing benefits while minimizing side effects. Pioneering examples of personalized therapies within oncology, such as trastuzumab for HER2-positive breast cancer cases and cetuximab for colorectal cancer associated with EGFR and KRAS mutations, illustrate the application of this approach [125].

18. Harnessing artificial intelligence in design of next generation of monoclonal antibody

The conventional process of developing new antibodies can be extensively time-consuming and expensive, which has led scientists to investigate the power of artificial intelligence (AI) to facilitate and hasten the discovery process. Therefore, there is an essential demand for establishing new computational and particularly ML tools for antibody design and discovery to obtain prompt, affordable, and customizable mAbs production [126]. One of the key benefits of AI in antibody development is its ability to analyze vast amounts of data from diverse sources rapidly. For example, AI algorithms can analyze high-throughput genomics data to identify novel disease targets and predict which targets will most likely respond to antibody therapy. Similarly, AI can analyze protein structures and predict which areas of a target protein are most accessible and vulnerable to antibody binding [127].

AI plays a key role in understanding antibody-antigen interfaces and learnability, optimizing antibody design factors like plasma half-life, and generating an infinitive set of novel antibodies with controlled properties that enhance or surpass those in the training databank [128]. For instance, AbDiver is a software that analyzes universally available next-generation sequencing information regarding B cell receptors and compares custom sequences with native repertoires. This software streamlines the investigation of vast antibody mutations to facilitate rational design and manipulation

of therapeutic antibodies. Likewise, antibody sequences from the PDB are deposited in the Structural Antibody Databank (SAbDab) comprising 12,367 Fv sequence structures as of June 2022, as well as abYsis. Moreover, SAbDab contains information on 748 therapeutic structures, including mAbs, bispecific therapeutics, and nanobody-related biotherapeutics, as of July 2022 [128, 129].

Jin et al. have introduced an algorithm that produces the sequence and conformation of a CDR-H3 loop in an autoregressive mode, focusing on typical antibody sequence engineering. Predicting the next residue is influenced by an initial illustration of the antibody framework and the current amino acids in the generated loop. The model creates presumptive forecasting for the following residue in the loop and can also generate counterpart 3D atomic peculiarities for the subsequent residue formed in the loop [130]. Recently, RFdiffusion and Chroma, both works according to score generative models, have successfully produced all-atom coordinates for new antibodies. Chroma is noteworthy for designing CDR portions in antibodies consisting of thousands of residues [131].

Novimmune® enhanced the construction of bispecific antibodies through codon optimization. Moreover, Rosenberg et al. demonstrated that calculating antibody conformation coordinates relies on codon frequency. This approach could lead to the development of DL algorithms. It has yet to be shown whether advancements in antibody conformation and sequence-based engineering can be combined into a seamless, differentiable process for producing epitope-conditioned antibodies without affecting the individual components' functionality [132].

19. Conclusion

The clinical antibody field has quickly developed during few late years and has been converted into the main power in the clinical market. Novel mAbs production techniques need a broad spectrum of DNA manipulation methods. Recent progress regarding novel engineering platforms associated with therapeutic antibody manufacturing has been summarized, such as chimeric antibodies, humanized versions, display technologies, transgenic animals, and single B cell technique, which have been key methods in the antibody development since the 1970s. While newer techniques such as transgenic animal and human single B cell methods have emerged, phage display remains valuable for its efficient and cost-effective selection process. Recent advancements such as high-throughput automatic screening and sequencing with next-generation and single-cell modalities are expected to enhance the identification of phage binders, speeding up monoclonal antibody production for research, diagnostics, and pharmaceutical treatment of human diseases.

Moreover, several of these technologies can be combined to effectively design and optimize therapeutic mAbs and Fc fusion proteins. Recently, significant technical advancements have focused on enhancing the antibody therapeutic effects of ADCC, ADCP, or CDC. This includes utilizing antibody Fc single mutations or glycoengineering to boost effector activities. Additional manipulations on antibodies to enhance therapeutic potential involve immune cytokines, ADCs, antibody-radionuclide conjugates, bispecific antibodies, immunoliposomes, and CAR-T/NK cell therapy. Future landscapes look hopeful in the framework of personalized medicine as antibodies are extensively utilized in clinics (designed for enhanced pharmacokinetics, pharmacodynamics, and decreased undesired immunological side effects), detection, and cancer immunotherapy in a subgroup of human population with similar genetic makeup.

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Author contribution

Mahdi Barazesh has been participated in work conceptual design, data gathering, writing, editing, and integrating the contents of complete manuscript. Shiva Mohammadi has been involved in writing some parts of manuscript. Sajad Jalili has helped in writing and editing through manuscript.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Author details

Mahdi Barazesh[1*], Shiva Mohammadi[2] and Sajad Jalili[3]

1 Department of Medical Biotechnology, School of Paramedical, Gerash University of Medical Sciences, Gerash, Iran

2 Hepatitis Research Center, Department of Medical Biotechnology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

3 Department of Orthopedics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

*Address all correspondence to: barazeshmahdi@yahoo.com

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References

[1] Pirkalkhoran S, Grabowska WR, Kashkoli HH, Mirhassani R, Guiliano D, Dolphin C, et al. Bioengineering of antibody fragments: Challenges and opportunities. Bioengineering. 2023;**10**(2):122

[2] Lu R-M, Hwang Y-C, Liu I-J, Lee C-C, Tsai H-Z, Li H-J, et al. Development of therapeutic antibodies for the treatment of diseases. Journal of Biomedical Science. 2020;**27**:1-30

[3] Goulet DR, Atkins WM. Considerations for the design of antibody-based therapeutics. Journal of Pharmaceutical Sciences. 2020;**109**(1):74-103

[4] Tian M, Cheng H-L, Kimble MT, McGovern K, Waddicor P, Chen Y, et al. An *in vivo* method for diversifying the functions of therapeutic antibodies. National Academy of Sciences of the United States of America. 2021;**118**(10):e2025596118

[5] Lundahl ML, Fogli S, Colavita PE, Scanlan EM. Aggregation of protein therapeutics enhances their immunogenicity: Causes and mitigation strategies. RSC Chemical Biology. 2021;**2**(4):1004-1020

[6] Zhang S, Wu L, Dang M. Antibody mimetics: The next generation antibody engineering, a retrospective and prospective analysis. Biotechnology Journal. 2024;**19**(1):e2300532

[7] Kadonosono T, Yimchuen W, Ota Y, See K, Furuta T, Shiozawa T, et al. Design strategy to create antibody mimetics harbouring immobilised complementarity determining region peptides for practical use. Scientific Reports. 2020;**10**(1):891

[8] Yamatsugu K, Katoh H, Yamashita T, Takahashi K, Aki S, Tatsumi T, et al. Antibody mimetic drug conjugate manufactured by high-yield *Escherichia coli* expression and non-covalent binding system. Protein Expression and Purification. 2022;**192**:106043

[9] Puccetti M, Pariano M, Schoubben A, Giovagnoli S, Ricci M. Biologics, theranostics, and personalized medicine in drug delivery systems. Pharmacological Research. 2024;**201**:107086

[10] Niesen J, Sack M, Seidel M, Fendel R, Barth S, Fischer R, et al. SNAP-tag technology: A useful tool to determine affinity constants and other functional parameters of novel antibody fragments. Bioconjugate Chemistry. 2016;**27**(8):1931-1941

[11] Mitra S, Tomar PC. Hybridoma technology; advancements, clinical significance, and future aspects. Journal, Genetic Engineering & Biotechnology. 2021;**19**(1):159

[12] Chen WC, Murawsky CM. Strategies for generating diverse antibody repertoires using transgenic animals expressing human antibodies. Frontiers in Immunology. 2018;**9**:460

[13] Zhao L, Wu Q, Song R, Yun Z. Genetic engineering antibody: Principles and application. IOP Conference Series: Materials Science and Engineering. IOP Publishing; 2019;**612**(2):022045

[14] Dai J-M, Zhang X-Q, Dai J-Y, Yang X-M, Chen Z-N. Modified therapeutic antibodies: Improving efficacy. Engineering. 2021;**7**(11):1529-1540

[15] Lyu X, Zhao Q, Hui J, Wang T, Lin M, Wang K, et al. The global landscape of

approved antibody therapies. Antibody Therapeutics. 2022;**5**(4):233-257

[16] Parray HA, Shukla S, Samal S, Shrivastava T, Ahmed S, Sharma C, et al. Hybridoma technology a versatile method for isolation of monoclonal antibodies, its applicability across species, limitations, advancement and future perspectives. International Immunopharmacology. 2020;**85**:106639

[17] Bates A, Power C. David vs. Goliath: The structure, function, and clinical prospects of antibody fragments. Antibodies. 2019;**8**:28

[18] Jin S, Sun Y, Liang X, Gu X, Ning J, Xu Y, et al. Emerging new therapeutic antibody derivatives for cancer treatment. Signal Transduction and Targeted Therapy. 2022;**7**(1):39

[19] Radke NV, Mohamed S, Brown RB, Ibrahim I, Chhablani J, Amin HV, et al. Review on the safety and efficacy of Brolucizumab for Neovascular age-related macular degeneration from major studies and real-world data. Asia-Pacific Journal of Ophthalmology (Philadelphia, PA). 2023;**12**(2):168-183

[20] Kang TH, Seong BL. Solubility, stability, and avidity of recombinant antibody fragments expressed in microorganisms. Frontiers in Microbiology. 2020;**11**:1927

[21] Mazaheri S, Talebkhan Y, Mahboudi F, Nematollahi L, Cohan RA, Mirabzadeh Ardakani E, et al. Improvement of Certolizumab Fab' properties by PASylation technology. Scientific Reports. 2020;**10**(1):18464

[22] Alsajjan R, Mason WP. Bispecific T-cell engagers and chimeric antigen receptor T-cell therapies in glioblastoma: An update. Current Oncology. 2023;**30**(9):8501-8549

[23] Hardiman G. Next-generation antibody discovery platforms. National Academy of Sciences of the United States of America. 2012;**109**(45):18245-18246

[24] Alfaleh MA, Alsaab HO, Mahmoud AB, Alkayyal AA, Jones ML, Mahler SM, et al. Phage display derived monoclonal antibodies: From bench to bedside. Frontiers in Immunology. 2020;**11**:1986

[25] Frenzel A, Schirrmann T, Hust M. Phage display-derived human antibodies in clinical development and therapy. MAbs. 2016;**8**(7):1177-1194

[26] Schneider CS, Bhargav AG, Perez JG, Wadajkar AS, Winkles JA, Woodworth GF, et al. Surface plasmon resonance as a high throughput method to evaluate specific and non-specific binding of nanotherapeutics. Journal of Controlled Release : Official Journal of the Controlled Release Society. 2015;**219**:331-344

[27] Ledsgaard L, Ljungars A, Rimbault C, Sørensen CV, Tulika T, Wade J, et al. Advances in antibody phage display technology. Drug Discovery Today. 2022;**27**(8):2151-2169

[28] Hattori T, Lai D, Dementieva IS, Montaño SP, Kurosawa K, Zheng Y, et al. Antigen clasping by two antigen-binding sites of an exceptionally specific antibody for histone methylation. National Academy of Sciences of the United States of America. 2016;**113**(8):2092-2097

[29] Brinkmann U, Kontermann RE. The making of bispecific antibodies. MAbs. 2017;**9**(2):182-212

[30] Kunamneni A, Ogaugwu C, Bradfute S, Durvasula R. Ribosome display technology: Applications in disease diagnosis and control. Antibodies (Basel, Switzerland). 2020;**9**(3):29

[31] Li R, Kang G, Hu M, Huang H. Ribosome display: A potent display technology used for selecting and evolving specific binders with desired properties. Molecular Biotechnology. 2019;**61**(1):60-71

[32] Nagumo Y, Fujiwara K, Horisawa K, Yanagawa H, Doi N. PURE mRNA display for *in vitro* selection of single-chain antibodies. The Journal of Biochemistry. 2016;**159**(5):519-526

[33] Jakobovits A. Therapeutic antibodies from XenoMouse transgenic mice. In: Little M, editor. Recombinant Antibodies for Immunotherapy. Cambridge: Cambridge University Press; 2009. pp. 89-99

[34] Pedrioli A, Oxenius A. Single B cell technologies for monoclonal antibody discovery. Trends in Immunology. 2021;**42**(12):1143-1158

[35] Sun H, Hu N, Wang J. Application of microfluidic technology in antibody screening. Biotechnology Journal. 2022;**17**(8):e2100623

[36] Sandomenico A, Sivaccumar JP, Ruvo M. Evolution of *Escherichia coli* expression system in producing antibody recombinant fragments. International Journal of Molecular Sciences. 2020;**21**(17):6324

[37] Sifniotis V, Cruz E, Eroglu B, Kayser V. Current advancements in addressing key challenges of therapeutic antibody design, manufacture, and formulation. Antibodies (Basel, Switzerland). 2019;**8**(2):36

[38] Zhang RY, Shen WD. Monoclonal antibody expression in mammalian cells. In: Chames P, editor. Antibody Engineering: Methods and Protocols. 2nd ed. Totowa, NJ: Humana Press; 2012. pp. 341-358

[39] Diamos AG, Hunter JGL, Pardhe MD, Rosenthal SH, Sun H, Foster BC, et al. High level production of monoclonal antibodies using an optimized plant expression system. Frontiers in Bioengineering and Biotechnology. 2020;**7**:472

[40] Hong M, Li T, Xue W, Zhang S, Cui L, Wang H, et al. Genetic engineering of baculovirus-insect cell system to improve protein production. Frontiers in Bioengineering and Biotechnology. 2022;**10**:994743

[41] Davydova EK. Protein engineering: Advances in phage display for basic science and medical research. Biochemistry (Moscow). 2022;**87**(1):S146-SS67

[42] Chiu ML, Goulet DR, Teplyakov A, Gilliland GL. Antibody structure and function: The basis for engineering therapeutics. Antibodies. 2019;**8**(4):55

[43] Safdari Y, Farajnia S, Asgharzadeh M, Khalili M. Antibody humanization methods - A review and update. Biotechnology & Genetic Engineering Reviews. 2013;**29**:175-186

[44] Apgar JR, Mader M, Agostinelli R, Benard S, Bialek P, Johnson M, et al. Beyond CDR-grafting: Structure-guided humanization of framework and CDR regions of an anti-myostatin antibody. MAbs. 2016;**8**(7):1302-1318

[45] Kim JH, Hong HJ. Humanization by CDR grafting and specificity-determining residue grafting. Methods in Molecular Biology (Clifton, NJ). 2012;**907**:237-245

[46] Marks C, Hummer AM, Chin M, Deane CM. Humanization of antibodies using a machine learning approach on large-scale repertoire data. Bioinformatics. 2021;**37**(22):4041-4047

[47] Fernández-Quintero ML, Heiss MC, Liedl KR. Antibody humanization—The influence of the antibody framework on the CDR-H3 loop ensemble in solution. Protein Engineering, Design and Selection. 2020;**32**(9):411-422

[48] Choi Y, Hua C, Sentman CL, Ackerman ME, Bailey-Kellogg C. Antibody humanization by structure-based computational protein design. MAbs. 2015;**7**(6):1045-1057

[49] Schrade A, Bujotzek A, Spick C, Wagner M, Goerl J, Wezler X, et al. Back-to-germline (B2G) procedure for antibody devolution. Antibodies. 2019;**8**(3):45

[50] Zinsli LV, Stierlin N, Loessner MJ, Schmelcher M. Deimmunization of protein therapeutics – Recent advances in experimental and computational epitope prediction and deletion. Computational and Structural Biotechnology Journal. 2021;**19**:315-329

[51] Choi Y, Verma D, Griswold KE, Bailey-Kellogg C. EpiSweep: Computationally driven reengineering of therapeutic proteins to reduce immunogenicity while maintaining function. Methods in Molecular Biology. 2017;**1529**:375-398

[52] Margreitter C, Mayrhofer P, Kunert R, Oostenbrink C. Antibody humanization by molecular dynamics simulations-in-silico guided selection of critical backmutations. Journal of Molecular Recognition : JMR. 2016;**29**(6):266-275

[53] Almagro JC, Daniels-Wells TR, Perez-Tapia SM, Penichet ML. Progress and challenges in the design and clinical development of antibodies for cancer therapy. Frontiers in Immunology. 2018;**8**:1751

[54] Tennenhouse A, Khmelnitsky L, Khalaila R, Yeshaya N, Noronha A, Lindzen M, et al. Computational optimization of antibody humanness and stability by systematic energy-based ranking. Nature Biomedical Engineering. 2024;**8**(1):30-44

[55] Krawczyk K, Kelm S, Kovaltsuk A, Galson JD, Kelly D, Trück J, et al. Structurally mapping antibody repertoires. Frontiers in Immunology. 2018;**9**:1698

[56] Collins AM, Watson CT. Immunoglobulin light chain gene rearrangements, receptor editing and the development of a self-tolerant antibody repertoire. Frontiers in Immunology. 2018;**9**:2249

[57] Ponomarenko N, Chatziefthimiou SD, Kurkova I, Mokrushina Y, Mokrushina Y, Stepanova A, et al. Role of κ→λ light-chain constant-domain switch in the structure and functionality of A17 reactibody. Acta Crystallographica Section D, Biological Crystallography. 2014;**70**(Pt. 3):708-719

[58] Mishra AK, Mariuzza RA. Insights into the structural basis of antibody affinity maturation from next-generation sequencing. Frontiers in Immunology. 2018;**9**:117

[59] Chan DTY, Groves MAT. Affinity maturation: Highlights in the application of *in vitro* strategies for the directed evolution of antibodies. Emerging Topics in Life Sciences. 2021;**5**(5):601-608

[60] Pires DE, Ascher DB. mCSM-AB: A web server for predicting antibody-antigen affinity changes upon mutation with graph-based signatures. Nucleic Acids Research. 2016;**44**(W1):W469-W473

[61] Kiyoshi M, Caaveiro JM, Miura E, Nagatoishi S, Nakakido M, Soga S,

et al. Affinity improvement of a therapeutic antibody by structure-based computational design: Generation of electrostatic interactions in the transition state stabilizes the antibody-antigen complex. PLoS One. 2014;**9**(1):e87099

[62] Reis PBPS, Barletta GP, Gagliardi L, Fortuna S, Soler MA, Rocchia W. Antibody-antigen binding interface analysis in the big data era. Frontiers in Molecular Biosciences. 2022;**9**:945808

[63] Arslan M, Karadag D, Kalyoncu S. Conformational changes in a Vernier zone region: Implications for antibody dual specificity. Proteins. 2020;**88**(11):1447-1457

[64] Mason DM, Friedensohn S, Weber CR, Jordi C, Wagner B, Meng SM, et al. Optimization of therapeutic antibodies by predicting antigen specificity from antibody sequence via deep learning. Nature Biomedical Engineering. 2021;**5**(6):600-612

[65] Makowski EK, Kinnunen PC, Huang J, Wu L, Smith MD, Wang T, et al. Co-optimization of therapeutic antibody affinity and specificity using machine learning models that generalize to novel mutational space. Nature Communications. 2022;**13**(1):3788

[66] Fernández-Quintero ML, Quoika PK, Wedl FS, Seidler CA, Kroell KB, Loeffler JR, et al. Comparing antibody interfaces to inform rational Design of new Antibody Formats. Frontiers in Molecular Biosciences. 2022;**9**:812750

[67] Marchand A, Bonati L, Shui S, Scheller L, Gainza P, Rosset S, et al. Rational Design of Chemically Controlled Antibodies and Protein Therapeutics. ACS Chemical Biology. 2023;**18**(6):1259-1265

[68] Arslan M, Karadağ D, Kalyoncu S. Protein engineering approaches for antibody fragments: Directed evolution and rational design approaches. Turkish Journal of Biology = Turk biyoloji dergisi. 2019;**43**(1):1-12

[69] Chen Z, Wang X, Chen X, Huang J, Wang C, Wang J, et al. Accelerating therapeutic protein design with computational approaches toward the clinical stage. Computational and Structural Biotechnology Journal. 2023;**21**:2909-2926

[70] Swindells MB, Porter CT, Couch M, Hurst J, Abhinandan KR, Nielsen JH, et al. abYsis: Integrated antibody sequence and structure-management, analysis, and prediction. Journal of Molecular Biology. 2017;**429**(3):356-364

[71] Santos J, Pujols J, Pallarès I, Iglesias V, Ventura S. Computational prediction of protein aggregation: Advances in proteomics, conformation-specific algorithms and biotechnological applications. Computational and Structural Biotechnology Journal. 2020;**18**:1403-1413

[72] Walsh I, Seno F, Tosatto SC, Trovato A. PASTA 2.0: An improved server for protein aggregation prediction. Nucleic Acids Research. 2014;**42**(Web Server Issue):W301-W307

[73] Kuriata A, Iglesias V, Pujols J, Kurcinski M, Kmiecik S, Ventura S. Aggrescan3D (A3D) 2.0: Prediction and engineering of protein solubility. Nucleic Acids Research. 2019;**47**(W1):W300-W3w7

[74] Navarro S, Ventura S. Computational methods to predict protein aggregation. Current Opinion in Structural Biology. 2022;**73**:102343

[75] Montanucci L, Capriotti E, Frank Y, Ben-Tal N, Fariselli P. DDGun: An untrained method for the prediction

of protein stability changes upon single and multiple point variations. BMC Bioinformatics. 2019;**20**(14):335

[76] Pandurangan AP, Ochoa-Montaño B, Ascher DB, Blundell TL. SDM: A server for predicting effects of. Nucleic Acids Research. 2017;**45**(W1):W229-Ww35

[77] Caldararu O, Blundell TL, Kepp KP. A base measure of precision for protein stability predictors: Structural sensitivity. BMC Bioinformatics. 2021;**22**(1):88

[78] Pucci F, Bernaerts KV, Kwasigroch JM, Rooman M. Quantification of biases in predictions of protein stability changes upon mutations. Bioinformatics. 2018;**34**(21):3659-3665

[79] Pascovici D, Wu JX, McKay MJ, Joseph C, Noor Z, Kamath K, et al. Clinically relevant post-translational modification analyses-maturing workflows and bioinformatics tools. International Journal of Molecular Sciences. 2018;**20**(1):16

[80] Dangi AK, Sinha R, Dwivedi S, Gupta SK, Shukla P. Cell line techniques and gene editing tools for antibody production: A review. Frontiers in Pharmacology. 2018;**9**:630

[81] Mason DM, Weber CR, Parola C, Meng SM, Greiff V, Kelton WJ, et al. High-throughput antibody engineering in mammalian cells by CRISPR/Cas9-mediated homology-directed mutagenesis. Nucleic Acids Research. 2018;**46**(14):7436-7449

[82] Parola C, Neumeier D, Friedensohn S, Csepregi L, Di Tacchio M, Mason DM, et al. Antibody discovery and engineering by enhanced CRISPR-Cas9 integration of variable gene cassette libraries in mammalian cells. MAbs. 2019;**11**(8):1367-1380

[83] van der Horst HJ, Nijhof IS, Mutis T, Chamuleau MED. Fc-engineered antibodies with enhanced Fc-effector function for the treatment of B-cell malignancies. Cancers. 2020;**12**(10):3041

[84] Liu R, Oldham RJ, Teal E, Beers SA, Cragg MS. Fc-engineering for modulated effector functions—Improving antibodies for cancer treatment. Antibodies. 2020;**9**(4):64

[85] Kellner C, Otte A, Cappuzzello E, Klausz K, Peipp M. Modulating cytotoxic effector functions by Fc engineering to improve cancer therapy. Transfusion Medicine and Hemotherapy : Offizielles Organ der Deutschen Gesellschaft fur Transfusionsmedizin und Immunhamatologie. 2017;**44**(5):327-336

[86] Wang L-F, Yu M. Epitope identification and discovery using phage display libraries: Applications in vaccine development and diagnostics. Current Drug Targets. 2004;**5**(1):1-15

[87] Caliandro R, Belviso DB, Aresta BM, de Candia M, Altomare CD. Protein crystallography and fragment-based drug design. Future Medicinal Chemistry. 2013;**5**(10):1121-1140

[88] Votapka L, Amaro RE. Multistructural hot spot characterization with FTProd. Bioinformatics. 2013;**29**(3):393-394

[89] Coales SJ, Tuske SJ, Tomasso JC, Hamuro Y. Epitope mapping by amide hydrogen/deuterium exchange coupled with immobilization of antibody, on-line proteolysis, liquid chromatography and mass spectrometry. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry. 2009;**23**(5):639-647

[90] Aggeler R, Capaldi R, Dunn S, Gogol E. Epitope mapping of monoclonal antibodies to the *Escherichia coli* F1 ATPase α subunit in relation to activity effects and location in the enzyme complex based on cryoelectron microscopy. Archives of Biochemistry and Biophysics. 1992;**296**(2):685-690

[91] Morris GE. Epitope mapping. Immunochemical Protocols. Totowa, NJ: Human Press; 2005;**295**:255-267

[92] Gershoni JM, Roitburd-Berman A, Siman-Tov DD, Freund NT, Weiss Y. Epitope mapping: The first step in developing epitope-based vaccines. BioDrugs. 2007;**21**:145-156

[93] Abbott WM, Damschroder MM, Lowe DC. Current approaches to fine mapping of antigen–antibody interactions. Immunology. 2014;**142**(4):526-535

[94] Davidson E, Doranz BJ. A high-throughput shotgun mutagenesis approach to mapping B-cell antibody epitopes. Immunology. 2014;**143**(1):13-20

[95] Huang J, Ru B, Dai P. Bioinformatics resources and tools for phage display. Molecules. 2011;**16**(1):694-709

[96] Tomasik J, Jasiński M, Basak GW. Next generations of CAR-T cells-new therapeutic opportunities in hematology? Frontiers in Immunology. 2022;**13**:1034707

[97] Zhang L, Meng Y, Feng X, Han Z. CAR-NK cells for cancer immunotherapy: From bench to bedside. Biomarker Research. 2022;**10**(1):12

[98] Zhu C, Wu Q, Sheng T, Shi J, Shen X, Yu J, et al. Rationally designed approaches to augment CAR-T therapy for solid tumor treatment. Bioactive Materials. 2024;**33**:377-395

[99] Liu H, Lei W, Zhang C, Yang C, Wei J, Guo Q, et al. CD19-specific CAR T cells that express a PD-1/CD28 chimeric switch-receptor are effective in patients with PD-L1–positive B-cell lymphoma. Clinical Cancer Research. 2021;**27**(2):473-484

[100] Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nature Reviews Clinical Oncology. 2020;**17**(3):147-167

[101] Moghanloo E, Mollanoori H, Talebi M, Pashangzadeh S, Faraji F, Hadjilooei F, et al. Remote controlling of CAR-T cells and toxicity management: Molecular switches and next generation CARs. Translational Oncology. 2021;**14**(6):101070

[102] Cooper ML, Choi J, Staser K, Ritchey JK, Devenport JM, Eckardt K, et al. An "off-the-shelf" fratricide-resistant CAR-T for the treatment of T cell hematologic malignancies. Leukemia. 2018;**32**(9):1970-1983

[103] Ren J, Liu X, Fang C, Jiang S, June CH, Zhao Y. Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. Clinical Cancer Research. 2017;**23**(9):2255-2266

[104] Śledź M, Wojciechowska A, Zagożdżon R, Kaleta B. *In situ* programming of CAR-T cells: A pressing need in modern immunotherapy. Archivum Immunologiae et Therapiae Experimentalis. 2023;**71**(1):18

[105] Rosenberg SA. Cell transfer immunotherapy for metastatic solid cancer—What clinicians need to know. Nature Reviews Clinical Oncology. 2011;**8**(10):577

[106] Tumeh PC, Koya RC, Chodon T, Graham NA, Graeber TG,

Comin-Anduix B, et al. The impact of *ex vivo* clinical grade activation protocols on human T cell phenotype and function for the generation of genetically modified cells for adoptive cell transfer therapy. Journal of Immunotherapy (Hagerstown, Md: 1997). 2010;**33**(8):759

[107] Lu H, Zhao X, Li Z, Hu Y, Wang H. From CAR-T cells to CAR-NK cells: A developing immunotherapy method for hematological malignancies. Frontiers in Oncology. 2021;**11**:720501

[108] Carlsten M, Childs RW. Genetic manipulation of NK cells for cancer immunotherapy: Techniques and clinical implications. Frontiers in Immunology. 2015;**6**:266

[109] Hoerster K, Uhrberg M, Wiek C, Horn PA, Hanenberg H, Heinrichs S. HLA class I knockout converts allogeneic primary NK cells into suitable effectors for "off-the-shelf" immunotherapy. Frontiers in Immunology. 2021;**11**:586168

[110] Phatarpekar PV, Lee DA, Somanchi SS. Electroporation of siRNA to silence gene expression in primary NK cells. In: Natural Killer Cells: Methods and Protocols. Vol. 1441. Clifton, NJ. 2016. pp. 267-276

[111] Gomes-Silva D, Srinivasan M, Sharma S, Lee CM, Wagner DL, Davis TH, et al. CD7-edited T cells expressing a CD7-specific CAR for the therapy of T-cell malignancies. Blood, The Journal of the American Society of Hematology. 2017;**130**(3):285-296

[112] Su S, Zou Z, Chen F, Ding N, Du J, Shao J, et al. CRISPR-Cas9-mediated disruption of PD-1 on human T cells for adoptive cellular therapies of EBV positive gastric cancer. Oncoimmunology. 2017;**6**(1):e1249558

[113] MacLeod DT, Antony J, Martin AJ, Moser RJ, Hekele A, Wetzel KJ, et al. Integration of a CD19 CAR into the TCR alpha chain locus streamlines production of allogeneic gene-edited CAR T cells. Molecular Therapy. 2017;**25**(4):949-961

[114] Nguyen DN, Roth TL, Li PJ, Chen PA, Apathy R, Mamedov MR, et al. Polymer-stabilized Cas9 nanoparticles and modified repair templates increase genome editing efficiency. Nature Biotechnology. 2020;**38**(1):44-49

[115] Kunert R, Reinhart D. Advances in recombinant antibody manufacturing. Applied Microbiology and Biotechnology. 2016;**100**(8):3451-3461

[116] Zhang H, Deng M, Pei F, Wang S, Ho M. Next-generation antibody therapeutics: Discovery, development and beyond: Highlights of the third annual conference of the Chinese antibody society. Antibody Therapeutics. 2019;**2**(4):99-107

[117] Nishimiya D, Mano T, Miyadai K, Yoshida H, Takahashi T. Overexpression of CHOP alone and in combination with chaperones is effective in improving antibody production in mammalian cells. Applied Microbiology and Biotechnology. 2013;**97**(6):2531-2539

[118] Ku SC, Ng DT, Yap MG, Chao SH. Effects of overexpression of X-box binding protein 1 on recombinant protein production in Chinese hamster ovary and NS0 myeloma cells. Biotechnology and Bioengineering. 2008;**99**(1):155-164

[119] Wang L-X, Tong X, Li C, Giddens JP, Li T. Glycoengineering of antibodies for modulating functions. Annual Review of Biochemistry. 2019;**88**(1):433-459

[120] Sacco SA, Tuckowski AM, Trenary I, Kraft L, Betenbaugh MJ, Young JD, et al. Attenuation of glutamine

synthetase selection marker improves product titer and reduces glutamine overflow in Chinese hamster ovary cells. Biotechnology and Bioengineering. 2022;**119**(7):1712-1727

[121] Gupta SK, Sharma A, Kushwaha H, Shukla P. Over-expression of a codon optimized yeast cytosolic pyruvate carboxylase (PYC2) in CHO cells for an augmented lactate metabolism. Frontiers in Pharmacology. 2017;**8**:463

[122] Zhou M, Crawford Y, Ng D, Tung J, Pynn AF, Meier A, et al. Decreasing lactate level and increasing antibody production in Chinese hamster ovary cells (CHO) by reducing the expression of lactate dehydrogenase and pyruvate dehydrogenase kinases. Journal of Biotechnology. 2011;**153**(1-2):27-34

[123] Fischer S, Marquart KF, Pieper LA, Fieder J, Gamer M, Gorr I, et al. miRNA engineering of CHO cells facilitates production of difficult-to-express proteins and increases success in cell line development. Biotechnology and Bioengineering. 2017;**114**(7):1495-1510

[124] Deepa Jatti P, Rakesh N. Personalised precision medicine - a novel approach for Oral cancer management. In: Gokul S, editor. Oral Cancer. Rijeka: IntechOpen; 2021. p. Ch. 4

[125] Garattini L, Padula A. Precision medicine and monoclonal antibodies: Breach of promise? Croatian Medical Journal. 2019;**60**(3):284-289

[126] Li L, Gupta E, Spaeth J, Shing L, Jaimes R, Engelhart E, et al. Machine learning optimization of candidate antibody yields highly diverse sub-nanomolar affinity antibody libraries. Nature Communications. 2023;**14**(1):3454

[127] Blanco-González A, Cabezón A, Seco-González A, Conde-Torres D, Antelo-Riveiro P, Piñeiro Á, et al. The role of AI in drug discovery: Challenges, opportunities, and strategies. Pharmaceuticals (Basel, Switzerland). 2023;**16**(6):891

[128] Akbar R, Bashour H, Rawat P, Robert PA, Smorodina E, Cotet TS, et al. Progress and challenges for the machine learning-based design of fit-for-purpose monoclonal antibodies. MAbs. 2022;**14**(1):2008790

[129] Młokosiewicz J, Deszyński P, Wilman W, Jaszczyszyn I, Ganesan R, Kovaltsuk A, et al. AbDiver: A tool to explore the natural antibody landscape to aid therapeutic design. Bioinformatics. 2022;**38**(9):2628-2630

[130] Gao W, Mahajan SP, Sulam J, Gray JJ. Deep learning in protein structural Modeling and design. Patterns. 2020;**1**(9):100142

[131] Kim J, McFee M, Fang Q, Abdin O, Kim PM. Computational and artificial intelligence-based methods for antibody development. Trends in Pharmacological Sciences. 2023;**44**(3):175-189

[132] Rosenberg AA, Marx A, Bronstein AM. Codon-specific Ramachandran plots show amino acid backbone conformation depends on identity of the translated codon. Nature Communications. 2022;**13**(1):2815

Chapter 4

Advances in Clinical Pharmacogenomics and Prevention of Severe Cutaneous Adverse Drug Reactions in the Era of Precision Medicine

Patompong Satapornpong, Lisa Vorasatit and Shoban John

Abstract

Severe cutaneous adverse drug reactions (SCARs), including drug reactions with eosinophilia and systemic symptoms (DRESS), Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), are rare but severe life-threatening adverse drug reactions. Although their incidence is rare, the mortality rates are as high as 10% for DRESS, 1–5% for SJS and 25–50% for TEN. Recent studies have suggested that *HLA* genes are associated with SCARs during treatment with causative medicines. The *HLA* gene is located on chromosome 6p21.1–21.3 and consists of *HLA* class I, II and III. Interestingly, *HLA*-pharmacogenomic markers influence these mechanisms of immunopathogenesis in culprit drug-induced SCARs. However, due to genetic differences at the population level, drug-induced SCARs are varied; thus, the specific pharmacogenomic markers for ethnicity might differ among populations. For instance, the *HLA-A*31:01* allele is associated with carbamazepine-induced SCARs in Europeans and Japanese individuals, while the *HLA-B*15:02* allele is associated with carbamazepine-induced SJS-TEN among Thais, Han Chinese, Taiwanese and Southeast Asians populations. Such differences pose a major challenge to preventing SCARs. Therefore, knowledge of the pharmacogenomics, mechanisms of immunopathogenesis and ethnic-specific genetic variation related to drug-induced SCARs is needed.

Keywords: precision medicine, pharmacogenomics, severe cutaneous adverse reactions, *human leukocyte antigen*, ethnicity

1. Introduction

Over the last decade, precision medicine has developed diagnostic methods and focused on delivering the right treatments to individual patients by the integration of big data, artificial intelligence, genetics, omics, pharma, cogenomics, and environmental and social factors [1, 2]. In addition, physicians, pharmacists, health systems, policymakers and patients have recognized advances in precision medicine and led

to powerful discoveries of genetic variations with interindividual differences [3]. Interestingly, the knowledge of pharmacogenomics has been associated with causative drug-induced severe cutaneous adverse reactions (SCARs) as a biomarker in clinical precision medicine and innovation for therapeutic decisions in many countries [4–7].

Severe cutaneous adverse reactions (SCARs) are a delayed type of T-cell-mediated adverse drug reaction and are a major cause of morbidity and life-threatening [8]. SCARs include drug-induced hypersensitivity syndrome (DIHS), drug reactions with eosinophilia and systemic symptoms (DRESS), hypersensitivity syndrome (HSS), Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and acute generalized exanthematous pustulosis (AGEP) [9–11]. Previous studies have suggested that drug-induced SCARs are genetically influenced *by human leukocyte antigen* (*HLA*), peptide and T lymphocytes [12–14]. Particularly, the function of *HLA* genes is to present antigen or peptide to T lymphocytes. Research on the *HLA* genes and phenotypes of drug-induced SCARs has confirmed the use of pharmacogenomic biomarkers for screening in many populations. Therefore, we focused on clinical pharmacogenomics and causative drug-induced severe cutaneous adverse reactions over the past decade. Moreover, we discuss the influence of *human leukocyte antigen* (*HLA*) genes related to pharmacogenomic markers, mechanisms of immunopathogenesis in drug-induced SCARs, and ethnic-specific genetic variation and provide a rationale for predicting clinical precision medicine and therapeutic decisions.

2. Severe cutaneous adverse drug reactions (SCARs): epidemiology, etiology and clinical manifestations

Severe cutaneous adverse drug reactions (SCARs) represent a collection of rare but potentially fatal dermatological conditions that have garnered increasing attention in both clinical and research settings. SCARs arising from drug use are linked to significant health issues, increased mortality rates, elevated healthcare expenses, and substantial challenges in drug development. Epidemiological studies have revealed that SCARs encompass a wide range of conditions, primarily Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN), drug reactions with eosinophilia and systemic symptoms (DRESS) and acute generalized acute pustulosis (AGEP) syndrome, which are characterized by a low incidence rate but a high mortality rate, making them a significant concern for healthcare providers [15, 16].

Although rare, according to a review article by Wen-Hung CHUNG and it impacts approximately 2% of patients admitted to hospitals, with an annual occurrence ranging from 2 to 7 cases per million for SJS/TEN and 1 in 1000 to 1 in 10,000 instances of exposure to the causative agents in DRESS [9]. However, the mortality rates for these conditions differ, with approximately 5–10% for SJS, 10–25% for SJS/TEN overlap, 25–50% for TEN, and 10% for DRESS [17–19]. The etiology of SCARs is multifactorial and involves a complex interplay of genetic predisposition, immune dysregulation, and exposure to specific medications, with a range of drugs implicated as potential culprits. Interestingly, this diversity of genetics in causal agents has made it complicated to predict and prevent SCARs in clinical practice.

Clinically, SCARs manifest with a spectrum of severe cutaneous and mucosal manifestations, including blistering, epidermal detachment, and mucous membrane involvement, frequently accompanied by systemic symptoms. Epidemiological trends, underlying etiological factors, and the clinical presentation of SCARs, shed

light on the difficulties and necessity of early diagnosis and treatment in managing these rare yet devastating drug-induced SCARs [11].

2.1 Steven-Johnson syndrome (SJS) and toxic epidermal necrolysis

Stevens-Johnson syndrome (SJS) and its more severe form the Toxic epidermal necrolysis (TEN) is a rare yet life-threatening dermatological condition characterized by severe cutaneous adverse reactions (SCARs) characterized by extensive skin detachment and mucous membrane involvement. SJS is was defined as skin detachment of less than 10% of the total body surface area (BSA). SJS/TEN overlapping involved skin detachment of 10–30% of the BSA (**Figure 1**), while TEN is considered detachment of greater than 30% of the total BSA [17, 20]. Furthermore, approximately 50–95% of SJS-TEN cases are related to medication exposure. Symptoms of SJS and TEN usually occur 4–28 days after exposure to drugs, such as antiepileptic drugs (AEDs), nonsteroidal anti-inflammatory drugs (NSAIDs), and specific antibiotics [21]. However, the incidence rate of SJS-TEN differs among ethnicities.

However, the prevalence in the East Asian population is greater than that in other populations (11). According to the data from the incidences reported from Korea during 2010–2013, SJS and TEN were 3.96–5.03 and 0.94–1.45, respectively [22]. The characteristics of SJS-TEN are painful blistering skin detachment, stinging eyes, malaise, fever, headache, sore throat and multiple internal organ involvement (cardiovascular, pulmonary, gastrointestinal, and genitourinary system) [21, 23]. Erythrodermic rash eruption first affects the face, upper torso, and proximal extremities. Erythematous, purpuric macules with irregular or dusky-red macules and atypical target lesions are the initial lesions identified (typically beginning to appear approximately 4–28 days after treatment initiation in drug) that develop into fluid-filled bullae and necrotic keratinocytes and epidermal separation from the dermis, referred to as a positive Nikolsky sign [24–26]. Furthermore, other factors that

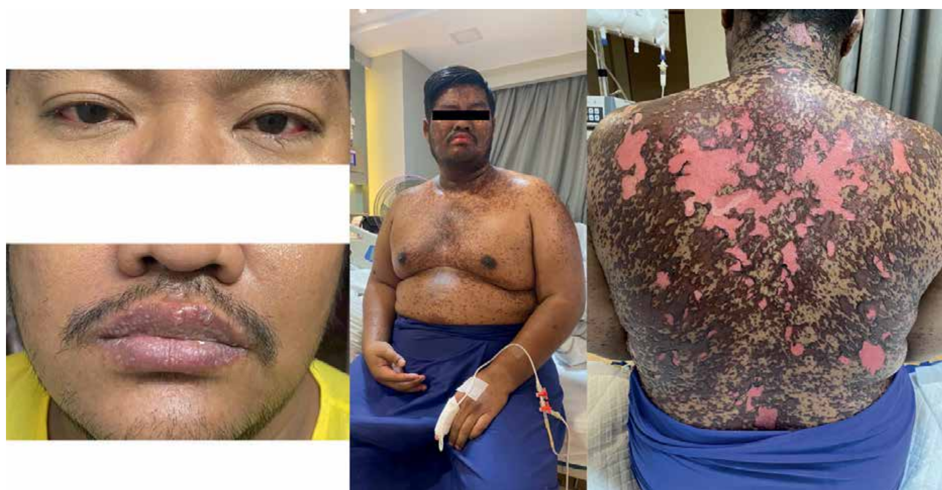

Figure 1.
Clinical presentations of Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) overlapping were performed by focusing on skin detachment of 10–30% of BSA. The prodromal phase is the first symptom and consists of fever, malaise (flu-like) and stinging eyes, sore throat and multiple internal organs.

increase the risk of developing SJS and TEN are HIV infection, *Mycoplasma pneumoniae* infection, other viruses (such as herpes simplex virus, HSV; cytomegalovirus, CMV; and human herpes virus 6, HHV-6) and hematologic malignancies [21]. The most significant factors contributing to mortality in the initial stages of the disease seem to be the severity of the illness, the onset of the reactions, advanced age, underlying disease and the number of skin detachment conditions, which are the primary factors associated with higher mortality rates.

Individuals who survive reactions such as SJS/TEN face a substantial risk of enduring complications that affect various bodily systems, including the dermatological system, eyes, mucous membranes, and respiratory, renal, and hepatic systems [27]. In light of these multifaceted challenges and the profound impact of SCARs on individuals and healthcare systems, there is a pressing need for continued pharmacogenomic research and clinical vigilance.

2.2 Drug reaction with eosinophilia and systemic symptoms (DRESS)

DRESS syndrome is a rare but potentially life-threatening drug-induced hypersensitivity syndrome distinguished by a collection of clinical symptoms, including fever (ranging from 38 to 40°C), widespread skin rash, facial edema, lymphadenopathy, hematological abnormalities (eosinophilia and atypical lymphocytes are usually found in more than 90 and 50% of cases, respectively) and the involvement of one or multiple organ systems, such as hepatitis, interstitial nephritis, myocarditis, pneumonitis and neurological involvement [19, 28]. Due to its extensive clinical presentation, DRESS rash is often polymorphic and includes maculopapular exanthema, which is the most common initial skin manifestation; purpuric, lichenoid, exfoliative, urticarial, and eczema-like lesions; blisters; and pustular lesions [29, 30]. In previous studies, we found that the estimated incidence of DRESS ranged from 1 in 1000 to 1 in 10,000 after drug exposure [31]. DRESS typically develops 2–8 weeks (average 22.2 days) after exposure to the culprit medication or many months after the drug has been discontinued, and the mortality rate is 10% [11, 32]. Additionally, the clinical presentation of DRESS was not significantly different between children and adults [30]. In clinical practice, the European Registry of Severe Cutaneous Adverse Reactions (RegiSCAR) scoring system has been used to establish diagnoses for DRESS syndrome [33]. Against this backdrop, the RegiSCAR scoring system has emerged as an indispensable tool in the field of DRESS syndrome diagnosis and classification. Its fundamental purpose is to stratify DRESS cases into four discrete tiers, namely, "no," "possible," "probable," or "definite" cases, based on a comprehensive evaluation of clinical presentation and laboratory findings with drug causality assessment [34, 35]. This systematic categorization not only refines the diagnostic process but also enhances our ability to differentiate DRESS from related severe cutaneous adverse reactions, a critical aspect of accurate patient management. Furthermore, the incidence of common culprit drug-induced DRESS caused by anticonvulsants has been reported. (phenytoin, carbamazepine, lamotrigine and phenobarbital), allopurinol, antibiotics (amoxicillin, ampicillin, azithromycin, levofloxacin, minocycline and vancomycin), sulfonamides (sulfamethoxazole-trimethoprim, dapsone and sulfasalazine) and antiviral drugs (abacavir and nevirapine) [11, 36]. Nevertheless, the impact of viral infection influences the pathophysiology, perturbation of the immune response and cause of DRESS syndrome caused by viruses such as human herpesvirus (HHV-6 and HHV-7), Epstein–Barr virus (EBV), cytomegalovirus (CMV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [31, 36, 37].

2.3 Acute generalized exanthematous pustulosis (AGEP)

Acute generalized exanthematous pustulosis (AGEP) is an uncommon severe adverse cutaneous reaction distinguished by the prompt emergence of numerous nonfollicular, aseptic pustules that are primarily located within the epidermal layer [38]. Furthermore, patients afflicted by AGEP typically exhibit accompanying clinical features, including fever (more than 38°C), leukocytosis (greater than 10,000 cells/mm^3), and neutrophilia (greater than 7000 cells/mm^3), which are commonly observed elements of this condition. Moreover, we detected clinical manifestations such as eosinophilia (approximately 30% of patients), hepatic dysfunction, renal failure, acute respiratory distress syndrome and lymphadenopathy [11, 39]. Compared with SJS-TEN and DRESS, AGEP is typically regarded as having a less severe clinical course, with a mortality rate less than 5% and an incidence of 1–5 patients per million per year [40, 41]. The onset of AGEP typically occurs within 24–48 hours after treatment starts with the causative drug [42].

Additionally, the most common medications that frequently triggered AGEP in the Asian population are penicillins, cephalosporins (ceftriaxone and cefuroxime), vancomycin and quinolones [40]. According to the data from spontaneous reports from 1984 to 2021 by the Health Product and Vigilance Center of Thailand, the culprit medications causing AGEP include ceftriaxone, clindamycin, ceftazidime, meropenem and amoxicillin-clavulanic acid. (https://hpvcth.fda.moph.go.th/spontaneous-2021/).

3. *Human leukocyte antigen* (HLA) gene and immune response

The human leukocyte antigen (HLA) is a substantial genetic entity, holding a pivotal position within the immune system. In humans, the HLA gene resides on the short arm of chromosome 6 (6p21.1–21.3), boasts an extensive repertoire of genes exceeding a count of 200 and belongs to the major histocompatibility complex (MHC) protein family [43, 44]. HLAs are classified by structure and function and are composed of HLA class I, II and III, as shown in **Figure 2** [45]. However, only two primary classes exist: HLA class I and HLA class II genes, which are strongly associated with drug-induced SCARs [46–48].

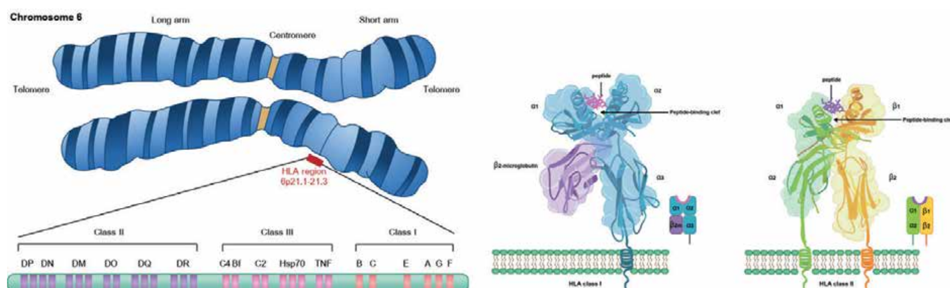

Figure 2.
Human leukocyte antigen (HLA) is a group of highly polymorphic genes located on chromosome 6p21.1–21.3. The HLA gene consists of HLA class I, II and III. In particular, HLA class I (HLA-A, HLA-B, and HLA-C) and II (HLA-DP, HLA-DQ and HLA-DR) genes were associated with drug-induced SCARs. The structure of HLA class I (comprising the alpha chain; α1, α2, α3 and beta-2 microglobulin; β2 m) and HLA class II (comprising the alpha chain; α1, α2 and the beta chain; β1, β2).

HLA class I molecules are located on nucleated cell surfaces and serve as media tors for presenting intracellular pathogen-derived antigens (e.g., viruses, certain bacteria, drugs) to cytotoxic T lymphocytes (CD8+ T lymphocytes) [49]. Their structure comprises a heavy chain (α-chain) consisting of three domains (α1, α2, and α3), with α1 and α2 forming a peptide-binding groove for antigenic peptide accommodation.

Beta-2 microglobulin (β2 m), a smaller non-HLA-encoded protein, associates with the α3 domain, ensuring HLA class I molecule stability (**Figure 2**). These molecules specifically bind short peptide antigens (usually 8–10 amino acids) from intracellular pathogens, which are inserted into the peptide-binding groove created by the α1 and α2 domains of the heavy chain [50]. The *HLA* class I alleles, which present molecules for CD8+ T lymphocytes, are further split into *HLA-A*, *HLA-B*, and *HLA-C* genes [51].

HLA class II molecules are primarily located on antigen-presenting cells (APCs), such as dendritic cells, macrophages, and B cells. They facilitate the presentation of antigens from extracellular pathogens to helper T lymphocytes (CD4+) [52]. HLA class II structures are more intricate than HLA class I structures and consist of two chains, the alpha chain (α-chain), which is encoded by HLA-D genes and features α1 and α2 domains that form a peptide-binding groove, and the beta chain (β-chain), which is encoded by the HLA-DP, HLA-DQ and HLA-DR genes and consists of the β1 and β2 domains (**Figure 2**). Peptide antigens sent by HLA class II are generally longer (typically 13–25 amino acids) and originate from extracellular pathogens digested by antigen-presenting cells [53]. Both HLA class I and class II molecules exhibit significant genetic diversity among individuals, enabling the immune system to recognize a broad array of pathogens. Recognition of antigens by T-cell receptors on T lymphocytes, based on HLA presentation, can initiate immune responses to eliminate infected cells or coordinate immune actions, depending on the type of T lymphocytes involved (CD8+ or CD4+) [54].

4. Mechanisms of immunopathogenesis in drug-induced SCARs

4.1 Hapten/ProHapten concept

In the late nineteenth century, experiments showed that small, nonimmunogenic molecules could become immunogenic when attached to larger carriers. Karl Landsteiner coined the term “hapten” in the early twentieth century while studying blood groups and these immunogenic compounds. By the mid-twentieth century, the hapten theory became crucial in understanding allergic reactions, explaining how haptens (drugs) create new antigenic determinants when bound to proteins. Today, this theory clarifies immune responses to various substances, including drugs and allergens, especially in drug-induced hypersensitivity reactions and SCARs. The process starts with exposure to a potential hapten-forming drug. Haptens, which are inherently nonimmunogenic small molecules, then covalently attach to endogenous proteins, forming drug-protein complexes (hapten-protein adducts) [55–57]. These complexes are considered foreign due to their drug or modified drug content. Immune cells, notably T lymphocytes, play has a vital role in recognizing foreign antigens, with T lymphocytes identifying antigenic peptides presented by human leukocyte antigen (HLA) molecules on cell surfaces, as presented in **Figure 3**. HLA molecules are responsible for presenting antigens to T lymphocytes. In drug-induced immune responses, HLA molecules present drug- protein complexes to T lymphocytes as antigens, especially in SCARs such as SJS and TEN. Cytotoxic CD8+

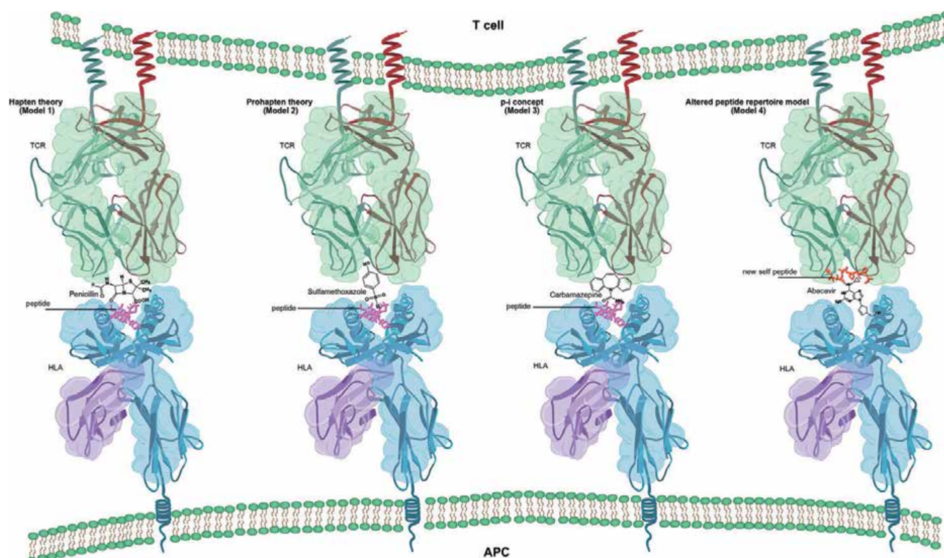

Figure 3.
Mechanisms of immunopathogenesis in drug-induced SCARs consisting of 4 theories of interactions between HLA, drugs, peptides and T lymphocytes: the Hapten theory (model 1), the Prohapten theory (model 2), the pharmacological interaction (p-i) concept (model 3) and the altered peptide repertoire model (model 4). APC, antigen-presenting cell; HLA, human leukocyte antigen; TCR, T-cell receptor.

T lymphocytes are often central to this process, leading to the destruction of skin and mucosal tissues and severe skin and mucosal reactions. Immune-mediated tissue damage is initiated by activated immune cells, mainly cytotoxic T lymphocytes, triggering inflammation in affected cells.

The prohapten theory extends the hapten theory, which proposes that certain drugs, referred to as prohapten, become immunogenic through metabolic transformations. These changes convert prohapten into highly reactive reactive intermediates that covalently bind to endogenous proteins, forming drug-protein complexes (hapten-protein adducts) [58, 59]. These complexes, considered foreign, contain the drug or its modified forms. T lymphocytes, which are receptors that recognize antigens presented by HLA molecules, are central to this process. In the prohapten.

In theory, HLA molecules present drug-protein complexes to T lymphocytes as antigens, potentially leading to various immune-related conditions, including severe cutaneous adverse drug reactions such as SJS and TEN.

4.2 Pharmacological interaction (p-i) concept

The p-i interaction theory elucidates how certain drugs or their metabolites directly engage the immune system, particularly immune cells such as T lymphocytes. These drugs or metabolites feature chemical structures known as "pharmacophores" capable of binding to specific sites on immune proteins or cell receptors (**Figure 3**). This interaction, which is typically noncovalent but potent, initiates an immune response involving various immune cells, such as T lymphocytes and B lymphocytes. Immune system activation by pharmacophores can result in immune-mediated effects, including inflammation, cellular damage, and hypersensitivity reactions.

HLA molecules play a role in the presentation of drug-derived antigens to CD4+ T lymphocytes (helper T lymphocytes), further activating immune responses.

This coordinated immune response can lead to diverse clinical outcomes, including allergic reactions and hypersensitivity [60–63].

4.3 Altered peptide repertoire model

In this mechanistic model, the drug engages in a noncovalent interaction with the HLA binding site, subsequently inducing a discernible shift in the chemical landscape of the binding cleft and the repertoire of endogenous peptides. This modification exerts a transformative influence on the selection and presentation of peptide ligands critical for the activation of T-cell receptors (TCRs) [64, 65]. The seminal research by Norcross et al. [66] underscores the noncovalent binding of abacavir to the HLA-B*57:01 molecule, instigating pronounced alterations in the peptide-binding capacity of the HLA-B*57:01 molecule, thereby affecting a profound transformation in the array of endogenous peptides made available for presentation to TCRs, as shown in **Figure 3**.

5. Pharmacogenomics of drug-induced SCARs

A deeper understanding of the immunopathogenesis mechanisms and *HLA* genes underpinning drug-induced SCARs, along with the identification of the pharmacogenomic markers in each ethnicity, is needed to improve the risk stratification of culprit drug-induced SCARs for the primary prevention and management of individual patients.

Carbamazepine (CBZ) is commonly prescribed for bipolar disorders, chronic or neuropathic pain and seizures [67], and other studies have shown that it induces patients to suffer from SJS and TEN. Some *HLA* alleles are commonly known as powerful predictive pharmacogenomic markers for SJS/TEN and DRESS in many populations. SJS/TEN is characterized by skin erythema, severe epidermal detachment, and mucous membrane disintegration. In Southeast Asian populations, the most pronounced marker from CBZ-induced SJS and TEN was found to be *HLA-B*15:02* [68]. In particular, the strong association between *HLA-B*15:02* and car- bamazepine-induced SJS/TEN in Han Chinese (odds ratio 2504, p value = 3.13×10^{-27}) [69], Malaysian (odds ratio 221.0, p value = 0.0006) [70], and Indian (odds ratio 71.40, p value = 0.0014) [71] and Thailand (odds ratio 54.43, p value = 2.89×10^{-12}) [72], as presented in **Table 1**. By comparison, *HLA-A*31:01* was the main genetic determinant for carbamazepine-induced SJS, TEN and DRESS in Japanese individuals (odds ratio 10.8, p value = 0.0004) [73] and Europeans (odds ratio 25.93, p value = 8.0×10^{-5}) and (odds ratio 12.41, p value = 0.03), respectively [74]. Previous studies by Rika Yuliwulandari et al. revealed that the *HLA-B*15:02* and *HLA-B*15:21* alleles in Indonesian patients were members of the *HLA-B/5* serotype, which was significantly associated with CBZ-induced SJS/TEN [80]. Oxcarbazepine (OXC) is an antiepileptic drug (AED) and a ketoanalog of CBZ. OXC blocks voltage-dependent sodium channels in the brain. Therefore, OXC is used for the treatment of partial seizures in patients who are unable to tolerate CBZ [81]. Similarly, based on the chemical structures of CBZ and OXC, SJS-TEN can be induced. Previous studies revealed that the cross-reactivity between carbamazepine-induced SJS-TEN and oxcarbazepine-induced SJS was associated with the *HLA-B*15:02* allele in an Asian population [75].

Allopurinol is a xanthine oxidase inhibitor and is commonly used for treatment of chronic gout, preventing tumor lysis syndrome (TLS) and preventing recurrent calcium nephrolithiasis in hyperuricosuria patients [82, 83]. However, allopurinol is one of the drugs most commonly associated with SJS and TEN [84]. Many studies

have shown that the *HLA-B*58:01* allele is strongly associated with allopurinol-induced SJS/TEN [85–88], which is similarly distributed in 7.38% of Asians (6.37%). Of African Americans, 6.38% of which were Thais, which was higher than that of Caucasians (1.13%) and Hispanics (1.07%) [89]. Research revealed an association between the *HLA-B*58:01* allele and allopurinol-induced SJS-TEN in Thai patients (odds ratio 579.0). p value <0.001) and allopurinol-induced DRESS (odds ratio 430.3,

Drugs	*HLA* markers	SCARs	Ethnic	OR (95% CI)	*p* value	References
Carbamazepine	*HLA- B*15:02*	SJS/TEN	Han Chinese	2504 (126–49,522)	3.13×10^{-27}	[69]
			Malaysian	221.0 (3.85–12,694.65)	0.0006	[70]
			Indian	71.40 (3.0–1698)	0.0014	[71]
			Thais	54.43 (16.28–181.96)	2.89×10^{-12}	[72]
	*HLA- A*31:01*	SCARs	Japanese	10.8 (5.9–19.6)	0.0004	[73]
		SJS-TEN	Europeans	25.93 (4.93–116.18)	8.0×10^{-5}	[74]
		HSS		12.41 (1.27–121.03)	0.03	
		MPE		8.33 (3.59–19.36)	8.0×10^{-7}	
Oxcarbazepine	*HLA- B*15:02*	SJS	Han Chinese	80.7 (3.8–1714.4)	8.4×10^{-4}	[75]
Allopurinol	*HLA- B*58:01*	SJS-TEN	Thais	579.0 (29.5–11,362.7)	< 0.001	[76]
		DRESS		430.3 (22.6–8958.9)	< 0.001	
		MPE		144.0 (13.9–1497.0)	< 0.001	
Abacavir	*HLA- B*5/:01*	ABC-HSRs	Europeans	29 (6.4–132.3)	< 0.0001	[77]
Cotrimoxazole	*HLA- B*15:02*	SJS-TEN	Thais	5.16 (1.63–16.33)	0.0075	[78]
	*HLA- B*13:01*	DRESS		15.20 (3.68–62.83)	7.2×10^{-5}	
Dapsone	*HLA- B*13:01*	DHS	Han Chinese	20.53 (11.55–36.48)	6.84×10^{-25}	[79]
		SCARs	Thais	39.00 (7.67–198.21)	5.3447×10^{-7}	
		SJS-TEN		36.00 (3.19–405.89)	2.1657×10^{-3}	[35]
		DRESS		40.50 (6.38–257.03)	1.0784×10^{-5}	

HLA-A, human leukocyte antigen-A; HLA-B, human leukocyte antigen-B; ABC-HSRs, abacavir hypersensitivity reactions; DRESS, drug reactions with eosinophilia and systemic symptoms; DHS, dapsone hypersensitivity syndrome; HSS, hypersensitivity syndrome; MPE, maculopapular exanthema; SCARs, severe cutaneous adverse reactions; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis. OR, odds ratio; 95% CI, 95% confidence interval; The p value and probability value were calculated using Fisher's exact test or the chi-square test.

Table 1.
The pharmacogenomic markers associated with drug-induced SCARs.

p value <0.001) and allopurinol-induced MPE (odds ratio 144.0, *p* value <0.001). Thus, *HLA-B*58:01* can be used as a universal pharmacogenomic marker for allopurinol-induced cutaneous adverse drug reactions (CADR) [76].

Abacavir (ABC) is a nucleoside reverse transcriptase inhibitor (NRTI) used to treat human immunodeficiency virus 1 (HIV-1) infection in both adults and children. However, abacavir hypersensitivity reactions (ABC-HSRs) are potentially life-threatening, with a mortality rate of 0.03% [90]. In addition to the clinical manifestations present within 6 weeks, ABC-HSRs usually develop in approximately 5–8% of patients after the initiation of treatment [91]. ABC-HSRs are a multiorgan process that occurs only in patients expressing *HLA-B*5/:01* [92]. A previous study reported associations between *HLA-B*5/:01* and ABC-HSRs in HIV-infected participants in Western Australia. The *HLA-B*5/:01* allele was present in 14 (78%) of the 18 participants with ABC-HSRs and in four (2%) of the 167 abacavir-tolerant participants (odds ratio 117, *p* value <0·0001) [93]. Furthermore, Dyfrig A. Hughes et al. reported a pooled odds ratio of 29 and a *p* value <0.0001 [77].

Cotrimoxazole (sulfamethoxazole and trimethoprim) is an antimicrobial used for the treatment and prophylaxis of *Pneumocystis jirovecii* pneumonia (PJP) in HIV patients and *toxoplasmic encephalitis patients* [94, 95]. Nonetheless, patients treated with cotrimoxazole reportedly develop cotrimoxazole-induced SCARs (SJS-TEN and DRESS). From 1984 to 2021, the Health Product and Vigilance Center of Thailand reported a list of causative medicines for causing SCARs in Thais, cotrimoxazole was the most common culprit drug causing SCARs, and SJS-TEN was the third most common culprit drug causing DRESS. Associations were found between the *HLA-B*15:02* allele and cotrimoxazole-induced SJS/TEN (odds ratio = 5.16, *p* value = 0.0075) and between the *HLA-B*13:01* allele and cotrimoxazole-induced DRSS (odds ratio = 15.20, *p* value = $7.2x10^{-5)}$ in Thai patients (https://hpvcth.fda.moph.go.th/spontaneous-2021/) [78]. Consequently, the *HLA-B*13:01* and *HLA-B*15:02* alleles are associated with co trimoxazole-induced DRESS and SJS-TEN, respectively.

Dapsone is widely used for treating infections (leprosy, *Pneumocystis jiroveci* pneumonia (PJP) and *Toxoplasma gondii* encephalitis in patients with HIV infection) and inflammatory disease. Moreover, dapsone-induced DRESS has an important influence on the mortality rate of 9.9% [96]. However, recently, many studies have investigated dapsone-induced DRESS in Thais and Han Chinese people. We found that the *HLA-B*13:01* allele was strongly associated with dapsone-induced DRESS (OR = 40.50, *p* value = 1.0784×10^{-5}) in the Thai population (OR = 20.53, *p* value = 6.84×10^{-25}) in Han Chinese [35, 79]. Moreover, the *HLA-B*13:01* allele was significantly associated with dapsone-induced SJS-TEN, with an OR of 36.00 ($p = 2.1657 \times 10^{-3}$) in Thai patients (**Table 1**).

6. Conclusion

Severe cutaneous adverse reactions (SCARs) are rare and life-threatening in many ethnicities. Notwithstanding the important role of *HLA* gene pharmacogenomic biomarkers related to drug-induced SCARs, precision medicine has improved the safety, efficacy and therapeutic decision-making process in this decade. Recent studies have shown that pharmacogenomic markers are effective at increasing the number of diagnoses and designing causative drug-induced SCAR protection and supporting clinical pharmacogenomic implementation.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Abbreviations

ABC-HSRs	abacavir hypersensitivity reactions
AED	antiepileptic drugs
AGEP	acute generalized exanthematous pustulosis
APCs	antigen-presenting cells
BSA	body surface area
CADR	cutaneous adverse drug reactions
CBZ	carbamazepine
CMV	cytomegalovirus
DIHS	drug-induced hypersensitivity syndrome
DRESS	drug reaction with eosinophilia and systemic symptoms
EBV	Epstein–Barr virus
HHV	human herpesvirus
HIV-1	human immunodeficiency virus 1
HLA	human leukocyte antigen
HSS	hypersensitivity syndrome
HSV	herpes simplex virus
MHC	major histocompatibility complex
MPE	maculopapular exanthema
NRTIs	nucleoside reverse transcriptase inhibitors
NSAIDs	nonsteroidal anti-inflammatory drugs
OXC	oxcarbazepine
p-i	pharmacological interaction
PJP	*pneumocystis jiroveci* pneumonia
RegiSCAR	registry of severe cutaneous adverse reactions SARS-CoV-2 severe acute respiratory syndrome coronavirus 2 (SCARs) severe cutaneous adverse drug reactions
SJS	Stevens–Johnson syndrome

SJS/TEN	overlap Stevens-Johnson syndrome/toxic epidermal necrolysis overlapping
TCR	T-cell receptor
TEN	toxic epidermal necrolysis
TLS	tumor lysis syndrome

Author details

Patompong Sataporpong[1,2]*, Lisa Vorasatit[3] and Shoban John[4,5]

1 Division of General Pharmacy Practice, Department of Pharmaceutical Care, College of Pharmacy, Rangsit University, Pathum Thani, Thailand

2 Excellence Pharmacogenomics and Precision Medicine Centre, College of Pharmacy, Rangsit University, Pathum Thani, Thailand

3 Queen's Medical Centre, The University of Nottingham Medical School, Nottingham, United Kingdom

4 Faculty of Medicine Ramathibodi Hospital, Division of Pharmacogenomics and Personalized Medicine, Department of Pathology, Mahidol University, Bangkok, Thailand

5 Laboratory for Pharmacogenomics, Somdech Phra Debaratana Medical Center (SDMC), Ramathibodi Hospital, Bangkok, Thailand

*Address all correspondence to: patompong.s@rsu.ac.th

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References

[1] Ramaswami R, Bayer R, Galea S. Precision medicine from a public health perspective. Annual Review of Public Health. 2018;**39**:153-168. DOI: 10.1146/annurev-publhealth-040617-014158

[2] Naithani N, Sinha S, Misra P, Vasudevan B, Sahu R. Precision medicine: Concept and tools. Medical Journal, Armed Forces India. 2021;**77**:249-257. DOI: 10.1016/j.mjafi.2021.06.021

[3] Wang WJ, Zhang T. Integration of traditional Chinese medicine and Western medicine in the era of precision medicine. Journal of Integrative Medicine. 2017;**15**:1-7. DOI: 10.1016/S2095-4964(17)60314-5

[4] Wang X. New strategies of clinical precision medicine. Clinical and Translational Medicine. 2022;**12**:1-3

[5] Hoffman JM, Dunnenberger HM, Hicks JK, Caudle KE, Carrillo MW, Freimuth RR, et al. Developing knowledge resources to support precision medicine: Principles from the clinical pharmacogenetics implementation consortium (CPIC). Journal of the American Medical Informatics Association. 2016;**23**:796-801. DOI: org/10.1093/jamia/ocw027

[6] Guin D, Rani J, Singh P, Grover S, Bora S, Talwar P, et al. Global text mining and development of pharmacogenomic knowledge resource for precision medicine. Frontiers in Pharmacology. 2019;**10**:1-11. DOI: 10.3389/fphar.2019.00839

[7] Bachtiar M, Sern Ooi BN, Wang J, Jin Y, Tan TW, Chong SS, et al. Toward precision medicine: Interrogating The human genome was used to identify drug pathways associated with potentially functional, population-differentiated polymorphisms. The Pharmacogenomics Journal. 2019;**19**:516-527. DOI: 10.1038/s41397-019-0096-y

[8] Peter JG, Lehloenya R, Dlamini S, Risma K, White KD, Konvinse KC, et al. Severe delayed cutaneous and systemic reactions to drugs: A global perspective on the science and art of current practice. The Journal of Allergy and Clinical Immunology. In Practice. 2017;**5**:547-563

[9] Chung WH, Wang CW, Dao RL. Severe cutaneous adverse drug reactions. The Journal of Dermatology. 2016;**43**:758-766. DOI: 10.1111/1346-8138.13430

[10] Gibson A, Deshpande P, Campbell CN, Krantz MS, Mukherjee E, Mockenhaupt M, et al. Updates on the immunopathology and genomics of severe cutaneous adverse drug reactions. The Journal of Allergy and Clinical Immunology. 2023;**151**:289-300. DOI: 10.1016/j.jaci.2022.12.005

[11] Tempark T, John S, Rerknimitr P, Satapornpong P, Sukasem C. Drug-induced severe cutaneous adverse reactions: Insights into clinical presentation, immunopathogenesis, diagnostic methods, treatment, and pharmacogenomics. Frontiers in Pharmacology. 2022;**13**:1-21. DOI: 10.3389/fphar.2022.832048

[12] Bharadwaj M, Illing P, Theodossis A, Purcell AW, Rossjohn J, McCluskey J. Drug hypersensitivity and human leukocyte antigens of the major histocompatibility complex. Annual Review of Pharmacology and Toxicology. 2012;**52**:401-431. DOI: 10.1146/annurev-pharmtox-010611-134701

[13] Pavlos R, Mallal S, Phillips E. HLA and pharmacogenetics of drug hypersensitivity. Pharmacogenomics. 2012;**13**:1285-1306. DOI: 10.2217/pgs.12.108

[14] Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides, and coreceptors. Annual Review of Immunology. 2006;**24**:419-466. DOI: 10.1146/annurev.23.021704.115658

[15] Sasidharanpillai S, Riyaz N, Khader A, Rajan U, Binitha MP, Sureshan DN. Severe cutaneous adverse drug reactions: A clinicoepidemiological study. Indian Journal of Dermatology. 2015;**60**:1-18. DOI: 10.4103/0019-5154.147834

[16] Guvenir H, Arikoglu T, Vezir E, Misirlioglu ED. Clinical phenotypes of severe cutaneous drug hypersensitivity reactions. Current Pharmaceutical Design. 2019;**25**: 3840-3854. DOI: 10.2174/1381612825666191107162921

[17] Hsu DY, Brieva J, Silverberg NB, Silverberg JI. Morbidity and mortality of Stevens-Johnson syndrome and toxic epidermal necrolysis in United States adults. The Journal of Investigative Dermatology. 2016;**136**:1387-1397. DOI: 10.1016/j.jid.2016.03.023

[18] Cao J, Zhang X, Xing X, Fan J. Biologic TNF-α inhibitors for Stevens–Johnson syndrome, toxic epidermal necrolysis, and TEN-SJS overlap: A study-level and patient-level meta-analysis. Dermatologic Therapy (Heidelb). 2023;**13**:1305-1327

[19] Hama N, Abe R, Gibson A, Phillips EJ. Drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS): Clinical features and pathogenesis. The Journal of Allergy and Clinical Immunology. In Practice. 2022;**10**:1155-1167. DOI: 10.1016/j.jaip.2022.02.004

[20] Letko E, Papaliodis DN, Papaliodis GN, Daoud YJ, Ahmed AR, Foster CS. Stevens-Johnson syndrome and toxic epidermal necrolysis: A review of the literature. Annals of Allergy, Asthma & Immunology. 2005;**94**:419-436. DOI: 10.1016/S1081-1206(10)61112-X

[21] Nates JL, Price KJ, editors. Oncologic Critical Care. 1st ed. Vol. 1. Switzerland AG: Springer Nature; 2019. DOI: 10.1007/978-3-319-74588-6_195

[22] Ma DH, Tsai TY, Pan LY, Chen SY, Hsiao CH, Yeh LK, et al. Clinical aspects of Stevens-Johnson syndrome/toxic epidermal necrolysis with severe ocular complications in Taiwan. Frontiers in Medicine (Lausanne). 2021;**8**:1-9. DOI: 10.3389/fmed.2021.661891

[23] Dunant AA, Mockenhaupt M, Naldi L, Correia O, Schröder W, Roujeau JC. Correlations between clinical patterns and causes of erythema multiforme majus, Stevens-Johnson syndrome, and toxic epidermal necrolysis: Results of an international prospective study. Archives of Dermatology. 2002;**138**:1019-1024. DOI: 10.1001/archderm.138.8.1019

[24] Hall JC, Hall BJ, editors. Cutaneous Drug Eruptions. 1st ed. Vol. 1. London: Springer-Verlag; 2015. DOI: 10.1007/978-1-4471-6729-7_24

[25] Chang HC, Wang TJ, Lin MH, Chen TJ. A review of the systemic treatment of Stevens-Johnson syndrome and toxic epidermal necrolysis. Biomedicine. 2022;**10**:1-16. DOI: 10.3390/biomedicines10092105

[26] White KD, Abe R, Jones MA, Beachkofsky T, Bouchard C, Carleton B,

et al. SJS/TEN 2017: Building multidisciplinary networks to drive science and translation. The Journal of Allergy and Clinical Immunology. In Practice. 2018;**6**:38-69. DOI: 10.1016/j.jaip.2017.11.023

[27] Lee HY, Walsh SA, Creamer D. Long-term complications of Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN): The spectrum of chronic problems in patients who survives an episode of SJS/TEN requires multidisciplinary follow-up. The British Journal of Dermatology. 2017;**177**:924-935. DOI: 10.1111/bjd.15360

[28] Cacoub P, Musette P, Descamps V, Meyer O, Speirs C, Finzi L, et al. The DRESS syndrome: A literature review. The American Journal of Medicine. 2011;**124**:588-597. DOI: 10.1016/j.amjmed.2011.01.017

[29] Martin G, Evan Lambert E, Wang GK. Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome caused by Apalutamide: A case presentation. Cureus. 2023;**15**:1-5. DOI: 10.7759/cureus.41687

[30] Manieri E, Dondi A, Neri I, Lanari M. Drug rash with eosinophilia and systemic symptoms (DRESS) syndrome in childhood: A narrative review. Frontiers in Medicine (Lausanne). 2023;**10**:1-11. DOI: 10.3389/fmed.2023.1108345

[31] Wang L, Mei XL. Drug reaction with eosinophilia and systemic symptoms: Retrospective analysis of 104 cases over one decade. Chinese Medical Journal. 2017;**130**:943-994. DOI: 10.4103/0366-6999.204104

[32] Choudhary S, McLeod M, Torchia D, Romanelli P. Drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome. The Journal of Clinical and Aesthetic Dermatology. 2013;**6**:31-37

[33] Kardaun SH, Sekula P, Allanore LV, Liss Y, Chu CY, Creamer D, et al. Drug reaction with eosinophilia and systemic symptoms (DRESS): An original multisystem adverse drug reaction. The results from the prospective study RegiSCAR study. The British Journal of Dermatology. 2013;**169**:1071-1080. DOI: 10.1111/bjd.12501

[34] Criado PR, Criado RF, Avancini JM, Santi CG. Drug reactions with eosinophilia and systemic symptoms (Dress)/drug-induced hypersensitivity syndrome (Dihs): A review of current concepts. Anais Brasileiros de Dermatologia. 2012;**87**:435-449. DOI: 10.1590/S0365-05962012000300013

[35] Satapornpong P, Pratoomwun J, Rerknimitr P, Klaewsongkram J, Nakkam N, Rungrotmongkol T, et al. HLA-B*13:01 is a predictive marker of Dapsone-induced severe cutaneous adverse reactions in Thai patients. Frontiers in Immunology. 2021;**12**:1-19. DOI: 10.3389/fimmu.2021.661135

[36] Ramirez GA, Ripa M, Burastero S, Benanti G, Bagnasco D, Nannipieri S, et al. Drug reaction with eosinophilia and systemic symptoms (DRESS): Focus on the pathophysiological and diagnostic role of viruses. Microorganisms. 2023;**11**:1-33. DOI: 10.3390/microorganisms11020346

[37] Descamps V, Rogez SR. DRESS syndrome. Joint, Bone, Spine. 2014;**81**:15-21. DOI: 10.1016/j.jbspin.2013.05.002

[38] Sidoroff A, Dunant A, Viboud C, Halevy S, Bouwes Bavinck JN, Naldi L, et al. Risk factors for acute generalized exanthematous

pustulosis (AGEP)-results of a multinational case–control study (EuroSCAR). The British Journal of Dermatology. 2007;**157**:989-996. DOI: 10.1111/j.1365-2133.2007.08156.x

[39] Ohz C, Allanore LV, Haddad C, Bouvresse S, Ortonne N, Duong TA, et al. Systemic involvement of acute generalized exanthematous pustulosis: A retrospective study on 58 patients. The British Journal of Dermatology. 2013;**169**:1223-1232. DOI: 10.1111/bjd.12502

[40] Qun Oh DA, Yeo YW, Lin Choo KJ, Pang SM, Oh CC, Lee HY. Acute generalized exanthematous pustulosis: Epidemiology, clinical course, and treatment outcomes of patients treated in an Asian academic medical center. JAAD International. 2021;**3**:1-6. DOI: 10.1016/j.jdin.2020.12.004

[41] Fernando SL. Acute generalized exanthematous pustulosis. The Australasian Journal of Dermatology. 2012;**53**:87-92. DOI: 10.1111/j.1440-0960.2011.00845.x

[42] Szatkowski J, Schwartz RA. Acute generalized exanthematous pustulosis (AGEP): A review and update. Journal of the American Academy of Dermatology. 2015;**73**:843-848. DOI: 10.1016/j.jaad.2015.07.017

[43] Choo SY, The HLA. System: Genetics, immunology, clinical testing, and clinical implications. Yonsei Medical Journal. 2007;**48**:11-23. DOI: 10.3349/ymj.2007.48.1.11

[44] Zakharova MY, Belyanina TA, Sokolov AV, Kiselev IS, Mamedov AE. The contribution of major histocompatibility complex class II genes to an association with autoimmune diseases. Acta Naturae. 2019;**11**:4-12. DOI: 10.32607/20758251-2019-11-4-4-12

[45] Sabbatino F, Liguori L, Polcaro G, Salvato I, Caramori G, Salzano FA, et al. Role of human leukocyte antigen system as a predictive biomarker for checkpoint-based immunotherapy in cancer patients. International Journal of Molecular Sciences. 2020;**21**:1-29. DOI: 10.3390/ijms21197295

[46] Kloypan C, Koomdee N, Satapornpong P, Tempark T, Biswas M, Sukasem C. A comprehensive review of HLA and severe cutaneous adverse drug reactions: Implication for clinical pharmacogenomics and precision medicine. Pharmaceuticals (Basel). 2021;**14**:1-34. DOI: 10.3390/ph14111077

[47] Matzaraki V, Kumar V, Wijmenga C, Zhernakova A. The MHC locus and genetic susceptibility to autoimmune and infectious diseases. Genome Biology. 2017;**18**:1-21. DOI: 10.1186/s13059-017-1207-1

[48] Kuruvilla R, Scott K, Pirmohamed M. Pharmacogenomics of drug hypersensitivity: Technology and translation. Immunology and Allergy Clinics of North America. 2022;**42**:335-355. DOI: 10.1016/j.iac.2022.01.006

[49] Wyatt RC, Lanzoni G, Russell MA, Gerling I, Richardson SJ. What the HLA-I!-classical and nonclassical HLA class I and their potential roles in type 1 diabetes. Current Diabetes Reports. 2019;**19**:1-11. DOI: 10.1007/s11892-019-1245-z

[50] Cruz-Tapias P, Castiblanco J, Anaya JM. Major histocompatibility complex: Antigen processing and presentation. In: Anaya JM, Shoenfeld Y, Rojas-Villarraga A, et al., editors. Handbook of Autoimmunity: From Bench to Bedside. 1st ed. Bogota (Colombia): El Rosario University Press; 2013. pp. 169-183

[51] Howell WM, Carter V, Clark B. The HLA system: Immunobiology, HLA typing, antibody screening and crossmatching techniques. Journal of Clinical Pathology. 2010;**63**:387-390. DOI: 10.1136/jcp.2009.072371

[52] Chen B, Khodadoust MS, Olsson N, Wagar LE, Fast E, Liu CL, et al. Predicting HLA class II antigen presentation through integrated deep learning. Nature Biotechnology. 2019;**37**:1332-1343. DOI: 10.1038/s41587-019-0280-2

[53] Wu Y, Zhang N, Hashimoto K, Xia C, Dijkstra JM. Structural comparison between MHC classes I and II; in evolution, a class-II-like molecule probably came first. Frontiers in Immunology. 2021;**12**:1-24. DOI: 10.3389/fimmu.2021.621153

[54] Wieczorek M, Abualrous ET, Sticht J, Álvaro-Benito M, Stolzenberg S, Noé F, et al. Major histocompatibility complex (MHC) class I and MHC class II proteins: Conformational plasticity in antigen presentation. Frontiers in Immunology. 2017;**8**:1-16. DOI: 10.3389/fimmu.2017.00292

[55] Carreno BM, Anderson RW, Coligan JE, Biddison WE. The HLA-B37 and HLA-A2.1 molecules bind largely nonoverlapping sets of peptides. Proceedings of the National Academy of Sciences of the United States of America. 1990;**87**:3420-3424. DOI: 10.1073/pnas.87.9.3420

[56] Waddington JC, Meng X, Illing P, Tailor A, Adair K, Whitaker P, et al. Identification of flucloxacillin-haptenated HLA-B*57:01 ligands: Evidence of antigen processing and presentation. Toxicological Sciences. 2020;**177**:454-465. DOI: 10.1093/toxsci/kfaa124.60

[57] Thomson P, Hammond S, Meng X, Naisbitt DJ. What's been Haptening? Over the last 88 years? Medicinal Chemistry Research. 2023;**32**:1950-1971. DOI: 10.1007/s00044-023-03091-1

[58] Pickard C, Smith AM, Cooper H, Strickland I, Jackson J, Healy E, et al. Investigation of mechanisms underlying the T-cell response to the hapten 2,4-dinitrochlorobenzene. The Journal of Investigative Dermatology. 2007;**127**:630-637. DOI: 10.1038/sj.jid.5700581

[59] Yun J, Cai F, Lee FJ, Pichler WJ. T-cell-mediated drug hypersensitivity: Immune mechanisms and their clinical relevance. Asia Pacific Allergy. 2016;**6**:77-89. DOI: 10.5415/apallergy.2016.6.2.77

[60] Zanni MP, von Grayerz S, Schnyder B, Brander KA, Frutig K, Hari Y, et al. HLA-restricted, processing- and metabolism-independent pathway of drug recognition by human alpha beta T lymphocytes. The Journal of Clinical Investigation. 1998;**102**:1591-1598. DOI: 10.1172/JCI3544

[61] Adam J, Pichler WJ, Yerly D. Delayed drug hypersensitivity: Models of T-cell stimulation. British Journal of Clinical Pharmacology. 2011;**71**:701-707. DOI: 10.1111/j.1365-2125.2010.03764.x

[62] Karnes JH, Miller MA, White KD, Konvinse KC, Pavlos RK, Redwood AJ, et al. Applications of immunopharmacogenomics: Predicting, preventing, and understanding immune-mediated adverse drug reactions. Annual Review of Pharmacology and Toxicology. 2019;**59**:463-486. DOI: 10.1146/annurev-pharmtox-010818-021818

[63] Yerly D, Pompeu YA, Schutte RJ, Eriksson KK, Strhyn A, Bracey AW, et al. Structural elements recognized by abacavir-induced T cells. International Journal of Molecular Sciences. 2017;**18**:1-10. DOI: 10.3390/ijms18071464

[64] Illing P, Vivian J, Dudek N, Kostenko L, Chen Z, Bharadwaj M, et al. Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. Nature. 2012;**486**:554-558. DOI: 10.1038/nature11147

[65] Ostrov DA, Grant BJ, Pompeu YA, Sidney J, Harndahl M, Southwood S, et al. Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:9959-9964. DOI: 10.1073/pnas.1207934109

[66] Norcross MA, Luo S, Lu L, Boyne MT, Gomarteli M, Rennels AD, et al. Abacavir induces loading of novel self- peptides into HLA-B*57: 01: An autoimmune model for HLA-associated drug hypersensitivity. AIDS. 2012;**26**:F21-F29. DOI: 10.1097/QAD.0b013e328355fe8f

[67] Ferrell PB Jr, McLeod HL. Carbamazepine, HLA-B*1502 and risk of Stevens-Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. Pharmacogenomics. 2008;**9**:1543-1546. DOI: 10.2217/14622416.9.10.1543

[68] Zhang Y, Wang J, Zhao LM, Peng W, Shen GQ, Xue L, et al. Strong association between HLA-B*1502 and carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in mainland Han Chinese patients. European Journal of Clinical Pharmacology. 2011;**67**:885-887. DOI: 10.1007/s00228-011-1009-4

[69] Chung WH, Hung SI, Hong HS, Hsih MS, Yang LC, Ho HC, et al. Medical genetics: A marker for Stevens-Johnson syndrome. Nature. 2004;**428**:486. DOI: 10.1038/428486a

[70] Then SM, Rani ZZ, Raymond AA, Ratnaningrum S, Jamal R. Frequency of The HLA-B*1502 allele contributes to carbamazepine-induced hypersensitivity reactions in a cohort of Malaysian epilepsy patients. Asian Pacific Journal of Allergy and Immunology. 2011;**29**:290-293

[71] Mehta TY, Prajapati LM, Mittal B, Joshi CG, Sheth JJ, Patel DB, et al. Association of HLA-B*1502 allele and carbamazepine-induced Stevens–Johnson syndrome among Indians. Indian Journal of Dermatology, Venereology and Leprology. 2009;**75**:579-582. DOI: 10.4103/0378-6323.57718

[72] Kulkantrakorn K, Tassaneeyakul W, Tiamkao S, Jantararoungtong T, Prabmechai N, Vannaprasaht S. Reported that HLA-B*1502 strongly predicts carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Thai patients with neuropathic pain. Pain Practice. 2012;**12**:202-208. DOI: 10.1111/j.1533-2500.2011.00479.x

[73] Ozeki T, Mushiroda T, Yowang A, Takahashi A, Kubo M, Shirakata Y, et al. Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in the Japanese population. Human Molecular Genetics. 2011;**20**:1034-1041. DOI: 10.1093/hmg/ddq537

[74] McCormack M, Alfirevic A, Bourgeois S, Farrell JJ, Kasperaviciute D, Carrington M, et al. HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. The New England Journal of Medicine. 2011;**364**:1134-1143. DOI: 10.1056/NEJMoa1013297

[75] Hung SI, Chung WH, Liu ZS, Chen CH, Hsih MS, Hui RC. Common risk allele in aromatic antiepileptic drug-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in

Han Chinese. Pharmacogenomics. 2010;**11**:349-356. DOI: 10.2217/pgs.09.162

[76] Sukasem C, Jantararoungtong T, Kuntawong P, Puangpetch A, Koomdee N, Satapornpong P, et al. HLA-B*58:01 for allopurinol-induced cutaneous adverse drug reactions: Implication for clinical interpretation in Thailand. Frontiers in Pharmacology. 2016;**7**:1-8. DOI: 10.3389/fphar.2016.00186

[77] Hughes DA, Vilar FJ, Ward CC, Alfirevic A, Park BK, Pirmohamed M. Cost-effectiveness analysis of HLA B*5701 genotyping in preventing abacavir hypersensitivity. Pharmacogenetics. 2004;**14**:335-342. DOI: 10.1097/00008571-200406000-00002

[78] Sukasem C, Pratoomwun J, Satapornpong P, Klaewsongkram J, Rerkpattanapipat T, Rerknimitr P, et al. Genetic association of cotrimoxazole-induced severe cutaneous adverse reactions is phenotype specific: HLA class I genotypes and haplotypes. Clinical Pharmacology and Therapeutics. 2020;**108**:1078-1089. DOI: 10.1002/cpt.1915

[79] Zhang FR, Liu H, Irwanto A, Fu XA, Li Y, Yu GQ, et al. HLA-B*13:01 and the dapsone hypersensitivity syndrome. The New England Journal of Medicine. 2013;**369**:1620-1628. DOI: 10.1056/NEJMoa1213096

[80] Yuliwulandari R, Kristin E, Prayuni K, Sachrowardi Q, Suyatna FD, Menaldi SL, et al. Association of the HLA-B alleles with carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis in the Javanese and Sundanese population of Indonesia: The important role of the HLA-B75 serotype. Pharmacogenomics. 2017;**18**:1643-1648. DOI: 10.2217/pgs-2017-0103

[81] Kalis MM, Huff NA. Oxcarbazepine, an antiepileptic agent. Clinical Therapeutics. 2001;**23**:680-700. DOI: 10.1016/s0149-2918(01)80019-9

[82] Raaju UR, Gosavi S, Sriharsha K. Allopurinol: Sorrow to the marrow. Journal of Family Medicine and Primary Care. 2020;**9**:2511-2513. DOI: 10.4103/jfmpc.jfmpc_249_20

[83] Alakel N, Middeke JM, Schetelig J, Bornhäuser M. Prevention and treatment of tumor lysis syndrome, and the efficacy and role of rasburicase. Oncotargets and Therapy. 2017;**10**:597-605. DOI: 10.2147/OTT.S103864

[84] Halevy S, Ghislain PD, Mockenhaupt M, Fagot JP, Bouwes Bavinck JN, Sidoroff A, et al. Allopurinol is the most common cause of Stevens-Johnson syndrome and toxic epidermal necrolysis in Europe and Israel. Journal of the American Academy of Dermatology. 2008;**58**:25-32. DOI: 10.1016/j.jaad.2007.08.036

[85] Somkrua R, Eickman EE, Saokaew S, Lohitnavy M, Chaiyakunapruk N. Association of HLA-B*5801 allele and allopurinol-induced Stevens Johnson syndrome and toxic epidermal necrolysis: A systematic review and meta-analysis. BMC Medical Genetics. 2011;**12**:1-10. DOI: 10.1186/1471-2350-12-118

[86] Hung SI, Chung WH, Liou LB, Chu CC, Lin M, Huang HP, et al. HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:4134-4139. DOI: 10.1073/pnas.0409500102

[87] Kaniwa N, Saito Y, Aihara M, Matsunaga K, Tohkin M, Kurose K. HLA-B locus in Japanese patients with

anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis. Pharmacogenomics. 2008;**9**:1617-1622. DOI: 10.2217/14622416.9.11.1617

[88] Lonjou C, Borot N, Sekula P, Ledger N, Thomas L, Halevy S, et al. A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. Pharmacogenetics and Genomics. 2008;**18**:99-107. DOI: 10.1097/FPC.0b013e3282f3ef9c

[89] Satapornpong P, Jinda P, Jantararoungtong T, Koomdee N, Chaichan C, Pratoomwun J, et al. Genetic diversity of HLA class I and class II alleles in Thai populations: Contribution to genotype-guided therapeutics. Frontiers in Pharmacology. 2020;**11**:1-22. DOI: 10.3389/fphar.2020.00078

[90] Carolino F, Santos N, Piñeiro C, Santos AS, Soares P, Sarmento A, et al. Prevalence of abacavir-associated hypersensitivity syndrome and HLA-B*5701 allele in a Portuguese HIV-positive population. Porto Biomedical Journal. 2017;**2**:59-62. DOI: 10.1016/j.pbj.2016.12.004

[91] Koech MK, Ali SM, Karoney MJ, Kigen G. Severe abacavir hypersensitivity reaction in a patient with human immunodeficiency virus infection: A case report. Journal of Medical Case Reports. 2022;**16**:1-5. DOI: 10.1186/s13256-022-03647-6

[92] Mallal S, Nolan D, Witt C, Masel G, Martin AM, Moore C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to the HIV-1 reverse transcriptase inhibitor abacavir. Lancet. 2002;**359**:727-732. DOI: 10.1016/s0140-6736(02)07873-x

[93] Hetherington S, Hughes AR, Mosteller M, Shortino D, Baker KL, Spreen W, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. Lancet. 2002;**359**:1121-1122. DOI: 10.1016/S0140-6736(02)08158-8

[94] Cockerill FR, Edson RS. Trimethoprim-sulfamethoxazole. Mayo Clinic Proceedings. 1991;**66**:1260-1269. DOI: 10.1016/s0025-6196(12)62478-1

[95] Béraud G, François SP, Foltzer A, Abel S, Liautaud B, Smadja D, et al. Cotrimoxazole for treatment of cerebral toxoplasmosis: An observational cohort study during 1994-2006. The American Journal of Tropical Medicine and Hygiene. 2009;**80**:583-587. DOI: 10.4269/ajtmh.2009.80.583

[96] Tempark T, Satapornpong P, Rerknimitr P, Nakkam N, Saksit N, Wattanakrai P, et al. Dapsone-induced severe cutaneous adverse drug reactions are strongly linked with HLA-B*13:01 allele in the Thai population. Pharmacogenetics and Genomics. 2017;**27**:429-437. DOI: 10.1097/FPC.0000000000000306

Chapter 5

3D Printing Technology in the Pharmaceutical Industry and Its Application in Drug Delivery in the Context of Personalized Medication

Pravin Admane, Sheetal Mane and Kuldeep Vinchurkar

Abstract

Manufacturing drug delivery systems using traditional processes is unsuitable for producing dosage forms tailored to individual patient needs. Traditional methods are labor-intensive, inflexible in dosing, and time-consuming. Consequently, there is a pressing need for healthcare industries to develop drug-delivery systems that provide personalized treatment. 3D printing technology, a revolutionary method, formulates customized doses with complex drug combinations. This novel technique involves depositing printing ink containing one or more drugs using software-based designs. 3D-printed dosage forms offer significant advantages over traditional manufacturing techniques, including the ability to tailor personalized dosage forms on demand, ensure accurate and precise drug dosing, and provide flexibility in shape and design according to market needs. The application of 3D printing techniques results in more patient-oriented outcomes, enhancing rational therapy with minimal side effects. Beyond its use in diagnostic fields like magnetic resonance imaging (MRI) and computed tomography (CT), 3D printing is also applied to develop implants, personalized medical prosthetics, and solid dosage forms and enables rapid throughput screening of new drug molecules on 3D-printed biological tissues.

Keywords: 3D printing, pharmaceutical dosage forms, drug delivery system, personalized medication, types of 3D printing technology

1. Introduction

The traditional approach of treatment on the basis of "one-size-fits-all" is not fully sufficient for bespoke treatment of patient. As per report given by National Health Service (NHS), the mass production of Pharmaceutical products formulated by traditional approach is less effective in approximately up to 70% patients [1]. Also, the dosage

forms prepared by the traditional approach of manufacturing makes patient non-compliant due to various reasons like low accuracy of dose and more frequent administration of drug specially in geriatric patients and patients with comorbidities. To overcome these problems, there is an urgent need of development of such a novel technology and novel drug-delivery systems, which can enable personalized medication for individuals so as to increase patient compliance during administration of drug doses. This phenomenon is also useful for the society where much more variances in living as well as environment, different intensities of illness, drug-drug, and drug-food interactions are observed. Therefore, it is very much important to fabricate such a dosage form which will be able to release a drug in desired release pattern as per patient's needs [2].

Three-dimensional printing technology is one of the revolutionary technologies in the healthcare sector including the pharmaceutical field where the treatment of the patient is done by tailoring the dose as per individual's requirement. This technology is also called as the "additive manufacturing technique," in which the dosage forms are being prepared layer by layer in various shapes and sizes to meet patient's needs [3]. The different imaging techniques or computer-aided design software (CAD) such as computed tomography (CT) or magnetic resonance imaging (MRI) are being utilized to create 3D structures or patterns fulfilling the accuracy and precision in doses of drug [3]. This pattern is especially advantageous for the administration of drugs to pediatric and geriatric patients, for whom inadequate doses and more probability of occurrence of adverse drug reactions are limitations with traditional drug-delivery systems [4]. This 3D printing technology can efficiently dispense low concentration of drug with precised control over the release of the drug for tailored drug therapy. This novel technique is based on a layer-by-layer deposition of drug in the pattern of 3D objects using different designs available in a digital platform. This technique was initially outlined in the start of 1990s used for rapid prototyping to develop solid entities by depositing the drug in a lot of in series pattern. More than 30 years ago, the technique has been used in combined research of optics, robotics, and chemistry for the development of 3D model comprising UV-cured resins [2].

The mechanism of a 3D printer involves a computer-aided software program to create a 3D object by depositing a drug material in ink form onto a substrate material. The technique includes firstly the ejection of the drug material from the printer head above the x-y axis to generate the base of the product. The printer then moves along the entire length through the z-axis, in which a liquid binder is ejected onto the generated base of the product. The physical parameter of the system is to be set so that the desired thickness is achieved. These steps are repeated to fabricate the object layer by layer as per command given from the computer software. The unbound substrate is then removed, assuring the completion of 3D printing of the object as per selected design. With this mechanism, the different pharmaceutical delivery systems can be created consistently by controlling the precised droplet size and also achieving the desired complex drug release pattern [5].

3D printing technology is majorly classified into seven categories, namely, material jetting, material extrusion, binder jetting, powder bed fusion, sheet lamination, vat photopolymerization, and directed energy deposition.

This technique is useful in healthcare services in various sectors from primary to secondary care, that is, community pharmacies to hospital wards. Dispensing of medicines in such areas offer improvement in easy access of medicines on urgent basis, minimize product wastage, and enhance discharge frequency. 3D printing technology is playing a very important role in personalized medications, where the dose of the drug can be tailored as per the need of the patient. The traditional approach of medications

sometimes may cause some complex reactions or contradictions in most of the patients, which might be due to difference in physiology, drug response, etc. [6]. Such contradictions may further show severe side effects in the patients. To minimize such adverse reactions, medications may be discontinued for such patients, which may further develop drug resistance and also wastage of the product. But these disadvantages can easily be overcome by the 3D-printed product, as it requires a small dose and is easily administered as a single dose. The 3D-printed product allows more accurate and precised dose to be administered to the patient at right time. These products are also advantageous by enhancing therapeutic efficacy, more safety, cost-effective, and improving patient compliance [7]. In the pharmaceutical sector, 3D printing technology is being utilized in the fabrication of various patient-specific drug-delivery systems like oro-dispersible films, controlled release dosage forms, self-emulsifying drug delivery system, polypills, microneedles, transdermal drug delivery systems, gastroretentive dosage forms, etc. The technique is best suitable in resource-constrained areas like emergency departments, disaster areas, first response units, and military operations, where products can be immediately manufactured to provide instant therapeutic treatment to the patient [8, 9]. The entities with variable diffusivity properties and various densities, complex molecular geometries, and combination of multi-drugs and non-drug components can be easily formulated. This technique is also being utilized to enhance the therapeutic efficacy of drugs like poorly water-soluble, potent drugs, peptides, etc. However, some precaution needs to be taken specially for heat-sensitive entities and incompatibility. To overcome these limitations, 3D techniques can be combined with novel drug-delivery system (NDDS) approach for advanced product output meeting patient needs [10]. The drugs with high cost and administered to patient in rare diseases can also be procured using 3D printing technique [3, 5]. This 3D printing technology is being widely used in different sectors benefited to consumers like dentistry, implants, oral drug-delivery formulations, medical devices, bioprinting tissues and organs, etc. The detailed application will be discussed further (**Figure 1**).

1.1 Benefits of 3D printing technology

1.1.1 Flexible design

The more complex drug combination designs can be easily prepared using computer-aided design software.

1.1.2 Rapid prototyping

Allows instant manufacturing of parts within hours.

1.1.3 Sustainability

It provides significant strength and mechanical resistance to dosage forms, which allows easy incorporation of various drugs in combination.

1.1.4 Print on demand

It requires less space for installation of equipment, minimal requirement of stock inventories and also offer the fabrication of dose on individual's demand.

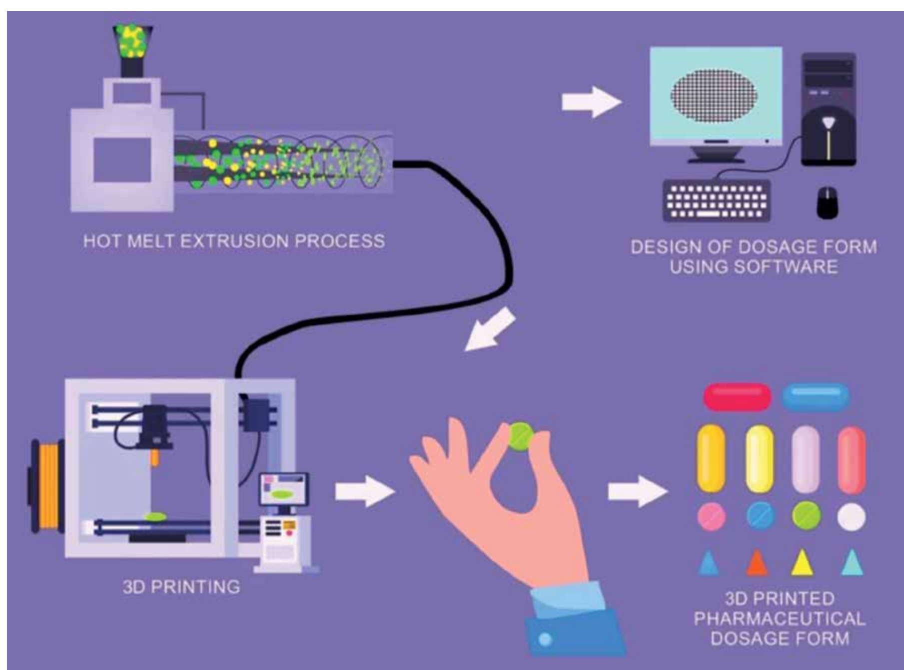

Figure 1.
3D printing technology in pharmaceutical dosage form.

1.1.5 Fast design and production

Computer-driven instructions are able to manufacture printed 3D objects very quickly with precised design and high reproducibility.

1.1.6 Minimal wastage

As ingredients required are very less and final composition is in liquid or semisolid form, the waste material remain after process is very minimum.

1.1.7 Cost effective

Due to single step process, it requires less amount of ingredients, minimal processing parameters, and less time; therefore, it is very cost-effective process.

1.1.8 Environmentally friendly

Because of lightweight parts of the equipment, the overall requirement of fuel to operate machine is low.

1.1.9 Advanced healthcare

The technique is having ability to print biological tissue, organs, complex molecular structures in a personalized dose very efficiently.

1.2 Disadvantages of 3D printing technology

1.2.1 Restricted build size

Large-sized particles cannot be printed through nozzles with small diameter.

1.2.2 Post processing

The parts of the machine are very delicate, so stringent precaution needs to be taken for cleaning after processing.

1.2.3 Large volumes

In case of mass production, no any cost per unit gets reduced.

1.2.4 Part structure

There may be a chance that the layers in 3D-printed objects get separated into distinct layers during processing.

1.2.5 Reduction in manufacturing jobs

As the whole 3D process is based on automation by computer-assisted software, the manpower required is less.

1.2.6 Design inaccuracies

Some printers require calibration of software at each time before printing to prevent inaccuracy in the design pattern.

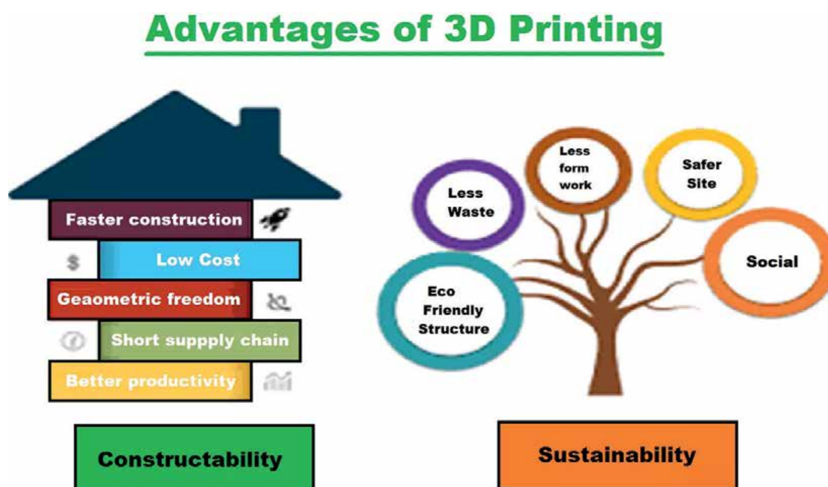

Figure 2.
Advantages of 3D printing technology.

1.2.7 Copyright issues

Easy access of this technology may lead to develop fake design or counterfeit products (**Figure 2**).

2. Classification of 3D printing technologies

The 3D printing technologies are being classified as follows (**Figure 3**).

2.1 Printing-based inkjet systems

This system involves deposition of liquid droplets onto the substrate to get products with desired release profile.

2.1.1 Continuous inkjet (CIJ) printing

In this technique, the ink comprising combinations of drug and excipients in different combinations is sprayed accurately in varying small-sized droplets layer by layer onto a non-powder-based substrate. CIJ printing technique produces a continuous stream of droplets in a pressurized flow. The system includes electrostatic plates which projects the droplets to be deposited onto the substrate. The waste material formed during process gets recirculated and used further (**Figure 4**) [11].

2.1.2 Drop on demand printing

This printing technique provides more precised object and minimal wastage of material. It consists approximate 1000 nozzles. The technique works on the principle of thermal and piezoelectric trigger systems. The process includes the generation of heat in thermal printhead by inducing electric current. This leads to the formation of bubble in the material to be printed. This causes the release out of small-sized liquid droplets from the nozzle which will then be deposited onto the substrate. This

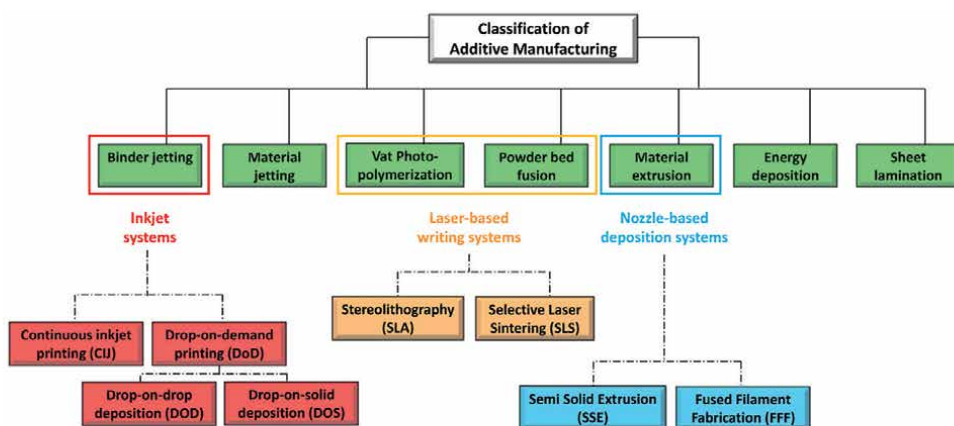

Figure 3.
Classification of 3D printing techniques.

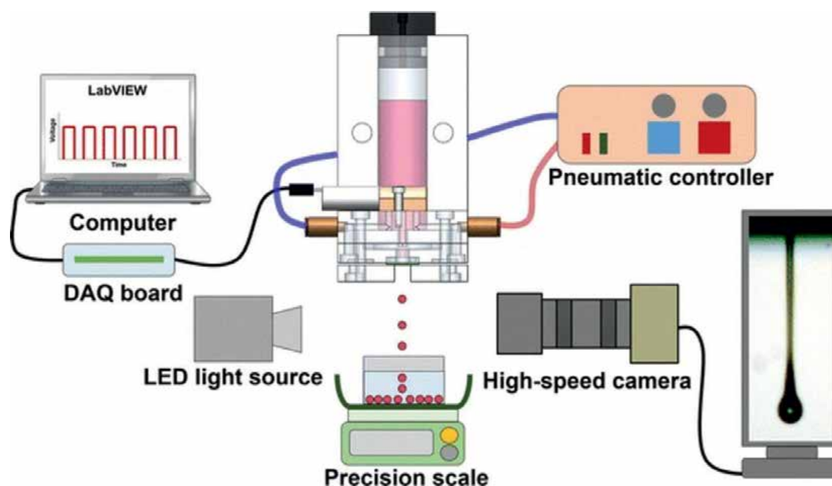

Figure 4.
Pneumatically controlled inkjet printer.

technique is unsuitable for heat-sensitive products as it produces high temperatures during working [12]. Next, piezoelectric printheads comprising piezoelectric materials expand and contract after the application of electrical current [13]. This expansion and contraction generate enough pressure required to eject a fluid in a small droplet with a shear rate of about 10^5 s^{-1} [7].

2.1.2.1 Drop on drop deposition

When the printed layer deposits over one another, it then creates a solidified layer of the product material. This process is called as drop on drop deposition.

2.1.2.2 Drop on solid deposition

When the droplets are being deposited onto solid material through the printer head is called as drop-on-solid deposition.

Both the systems offer advantages in a way that they are having ability to provide multiple depositions of ink at the same time using varying materials.

2.2 Laser-based writing systems

It works on the principle of photopolymerization, in which free radicals are being released out when the ultraviolet light beam is passed through the surface of the liquid resin to the previously designed 3D model.

2.2.1 Stereolithography (SLA)

In this technique, ultraviolet light is passed through the liquid surface, where the x-y axis is exposed to the formation of a distinct layer. This will then be modified as the z-axis as building process is evolved incrementally. When a previously deposited layer gets solidified, a fresh layer of liquid resin is again deposited in such a way that it develops a 3D-printed object. The process is continued till the product is completely

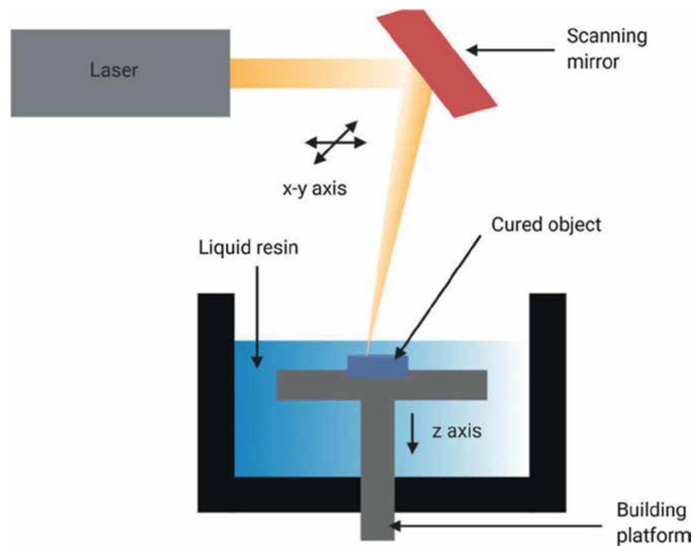

Figure 5.
Stereolithographic printer.

formed. The excess amount of resin is then being removed for recirculation, once the process is complete. Further UV flood post-curing step may be carried out if conversion of photopolymer is required. This step depends on the type of resin used. The roughness of the finished products is then prevented by using sealants, metallic coatings, etc. (**Figure 5**) [14].

2.2.2 Selective laser sintering (SLS)

This technique involves the use of thermoplastic polymers as the main feed material [9]. The laser beam is passed through the powder particles, which melts and fuses them together, and so the process is termed as "sintering." The very low power laser is required to heat the feed powder, which causes a small increase in temperature over the surface of the powder, and ultimately helps to induce sintering [15]. The apparatus consists of six parts: (i) a building platform, on which fabrication of 3D object is done; (ii) a laser for the sintering process; (iii) Galvano mirrors, used to project and direct the laser beam to the accurate printing positions; (iv) a powder reservoir, which holds and then dispenses fresh powder on the building platform; (v) a mechanical roller to spread out and to flatten the powder on the platform; (vi) a material vat to recover unsintered powder. First, the building platform is up to its uppermost position, on which a fresh layer of powder is spread and then flattened with the help of the roller. The laser beam is then activated, which scans and sinters the powder as per instructions given from the file to create the 3D pattern. The platform is then lowered and again creates enough space for a layer of fresh new powder. The powder reservoir platform then ascends, and the new layer of powder then spreads and flattened with the help of the roller. This process is repeated till the completion of the printing job. After completion, the printer is removed and kept for cooling. Excess amount of unsintered material is then being removed or cleaned with the help of compressed air and later on, and the printed object is then recovered (**Figure 6**) [16].

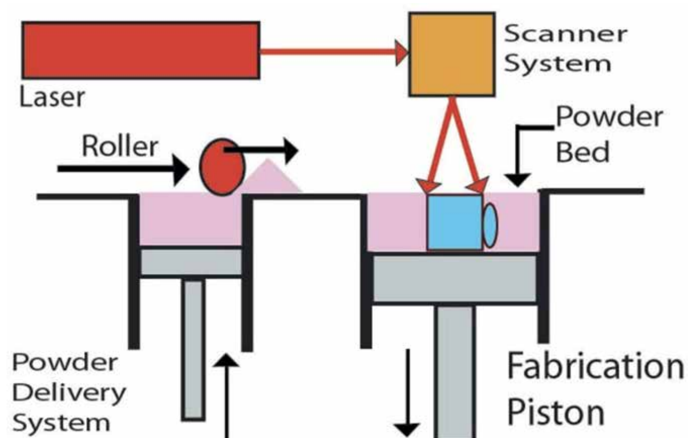

Figure 6.
Selective laser sintering.

2.3 Nozzle-based printing

This technique involves deposition of ink through the nozzle layer by layer onto the substrate as per the command given by computer aided software.

2.3.1 Semi-solid extrusion (SSE)

In this technique, ink comprising drug material is prepared which is dispensed through a nozzle using mechanical or pneumatic pressure [10]. This technique also forms layer by layer print to create a 3D object. The ink is prepared using a suitable polymer or hydrogel to get desired viscosity. The ink is then filled in plastic or metallic syringes and then dispensed in the form of droplets onto the substrate fixed on a building platform with the help of pneumatic pressure or piston-driven or screw-driven force [17]. Pneumatic systems work by using the compressed air and is more useful to dispense highly viscous polymer very efficiently. The piston-driven system causes deposition of the ink by controlling the flow of the hydrogel through the nozzle. On the other hand, screw-based systems provide more specific control for deposition of highly viscous hydrogels [18]. The extrusion-based printing provides maximum resolution up to 200 mm, but comparatively less than laser or inkjet-based printing (**Figure 7**).

2.3.2 Fused filament fabrication

Fused filament fabrication is also known as fused diffusion modeling (FDM). In this technique, the drug is first uniformly mixed in thermoplastic polymers, later on kept in a suitable solvent for incubation [19]. Both the drugs and thermoplastic polymers are melted together at required temperature with the help of hot-melt extrusion technique to get a desired molten mass. With the help of high-temperature nozzle, the molten mass is extruded into the filament. The layers formed after extrusion are immediately deposited onto a plate and solidified instantly. The current technique is capable to manufacture the production using highly complex drugs with difficult structures and geometries. It also provides good mechanical strength.

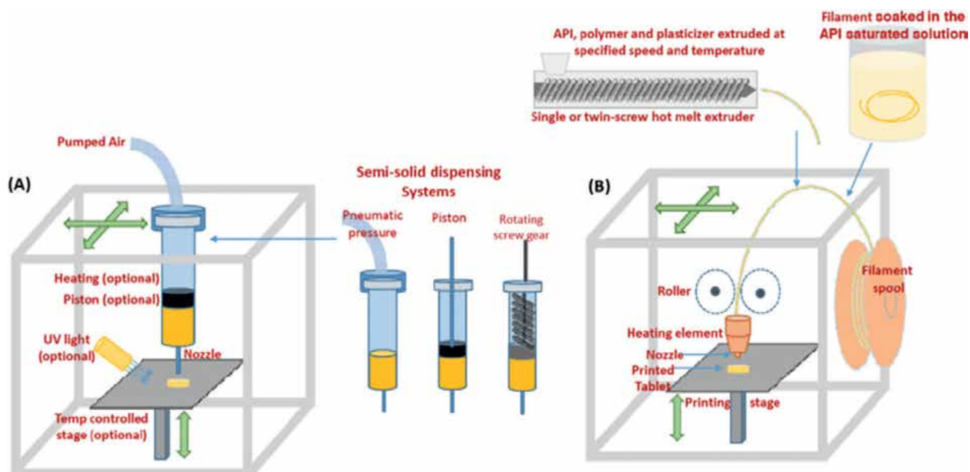

Figure 7.
(a) Semi-solid extrusion 3D printing technique. (b) Fused-diffusion modelling 3D printing technique.

In addition to this, this technique is also able to produce modified release 3D-printed object [20]. This extrusion-based technology has also been used to design diversified drug-delivery systems to get the desired release profile of the drug. Along with these several advantages in pharmaceutical applications, some drawbacks are existing with

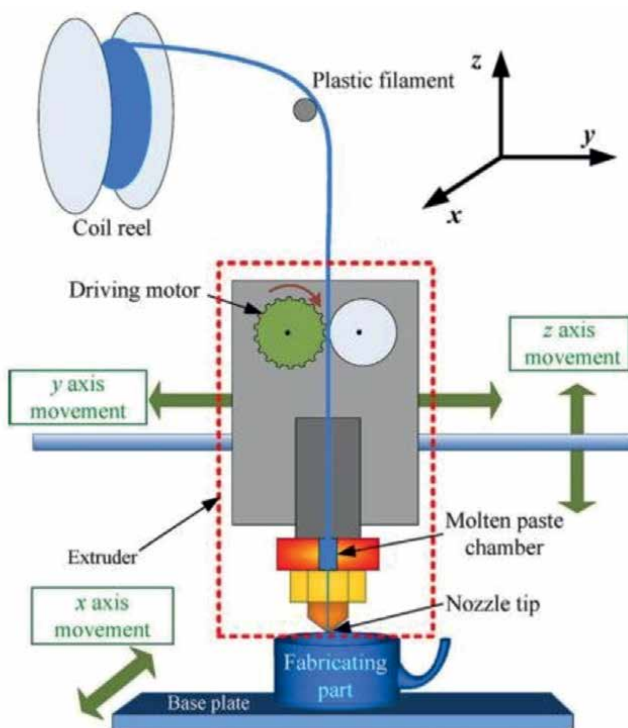

Figure 8.
Fused diffusion modeling.

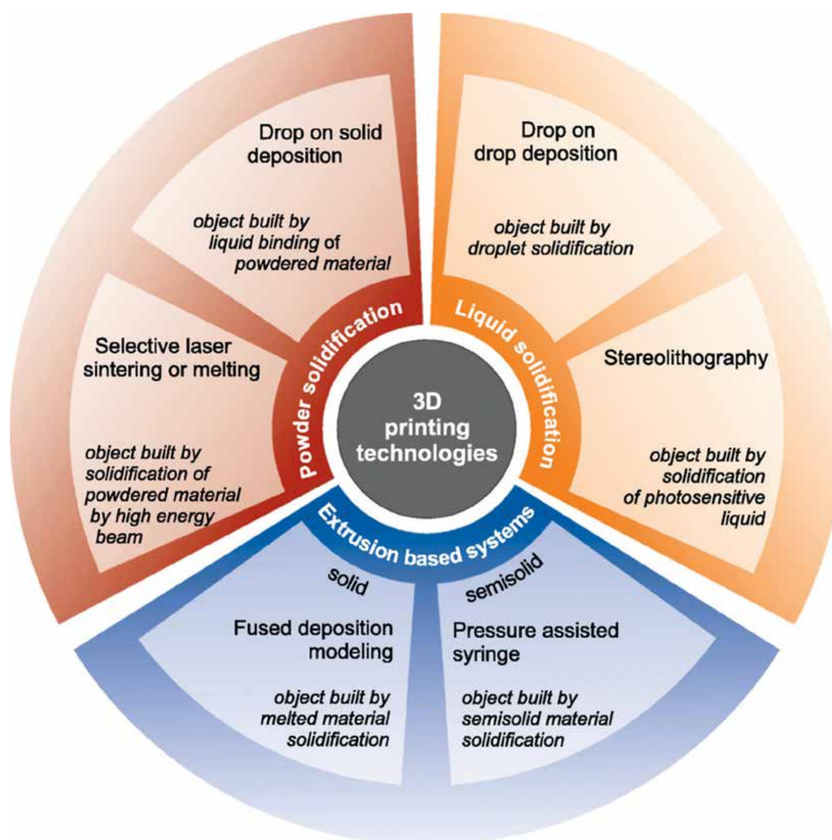

Figure 9.
3D printing technologies based on solid, liquid, and semisolid printing.

this process like lack of quantity of biodegradable thermoplastic polymers possessing good melted viscosity characteristics for their extrusion, high temperature used for extrusion not suitable for thermosensitive drugs (**Figures 8** and **9**) [20].

3. Applications of 3D printing technology in pharmaceutical sector

3.1 Personalized drug dosing

3D printing technique is useful to develop tailored dosage forms of very complex geometries [21]. It also helps to create object with modified release profile using a software instruction or command. The flexible drug delivery comprising a wide range of doses can be easily fabricated using this technique, especially for the pediatric and geriatric population. The printed film can also be easily prepared for the patients having swallowing problems.

Pietrzak et al. prepared a tablet using fused deposition modeling (FDM) 3D printing consisting of a flexible dose of theophylline for extended as well as immediate release profile with accuracy in drug loading capability and made a tablet design which is easily swallowed. The manufacturing process was easily controlled with the help of software and also was helpful to identify the compatibility of polymers being

used including Eudragit RS, RL, hydroxy-propyl cellulose, etc. With this technique, a dose accuracy was obtained up to 95% with a desired release profile. This method has ability to develop personalized dosage form for individual treatment in future [22]. Melocchi with his team developed the hot melt extrusion technique coupled with 3D printer to formulate printing filaments [23]. This hot melt extrusion is combined with fused deposition modeling to form extruded filaments of indomethacin. The polymers like hydroxypropyl methylcellulose and polyethylene glycol were added to form the filaments. Starmix, a flavored gummy sweet, was developed as chewable tablets using the 3D printing technique, which improved palatability and patient compliance.

Tagami et al. formulated curcumin tablets using polyvinyl alcohol as a polymer by fused deposition modeling. The factors like flow rate of polymer and printing temperature can affect the formation of the final product [15]. Such personalized 3D printed medications are also helpful to administer medicines with narrow therapeutic indices. The patients with a pharmacogenetic polymorphism can also be easily treated with these 3D-printed products. Wallis M et al. developed directly compressible 3D printed tablet using FDM based 3D printing [24]. The two important fundamental parameters pharmacogenomics and pharmacogenetics play a very important role in the development of personalized dosage form, as there is a need of tailoring personalized medication in case of patient with different phenotypes. The dose for them can be adjusted with printing technique [25]. The patients with comorbities and having multiple diseases can also be treated using personalized medication therapy by providing accurate and precised dose as per need.

3.2 Complex drug-release profiles

Manufacturing of dosage forms with complex drug release profiles is one of the critical approaches, but beneficial for the patients. Several dosage forms were being prepared with immediate release, sustained release, and delayed release profile by various scientists [26]. Development of guaifenesin tablets with sustained release profile using 3D printing technology, showed required dissolution profile with satisfaction of all regulatory aspects. In this, polyacrylic acid and hydroxypropyl methylcellulose were used as a hydrophilic polymer to get sustained release layer. The dosage forms of immediate release profile were also formulated using HPMC as a binding agent along with microcrystalline cellulose and sodium starch glycolate (SSG) as disintegrants [27].

Wang and team formulated a tablet with modified drug release using stereolithographic 3D printing and evaluated its suitability and stability. The tablets comprising paracetamol and 4-aminosalicylic acid as model drugs were prepared with required drug loading and obtained desired extended drug release profile [17]. This technique can be used to develop various personalized delivery systems like implants, 3D printed tablets, transdermal delivery system, etc [28]. Yu et al. developed tablets of acetaminophen showing zero order release with the help of 3D printing technique. Dissolution studies showed the drug release by the mechanism of two-dimensional surface erosion. From the study, it was found that the technology can be applicable to produce extended release profile by incorporation of extended release polymers and relevant ingredients [29]. Later on, Skowyra used fused deposition modeling 3D printing technique for the formulation of prednisolone tablets with extended release profile and found the desired and accurate results as expected.

Furthermore, Goyanes with his associates developed modified-release tablets of 4-aminosalicylic acid and 5-aminosalicylic acid using fused deposition modeling and

found that the tablets prepared were mechanically strong with desired release characteristics. They also found that the technique might not be able to fabricate the dosage forms of thermolabile drugs as thermal degradation of 4-aminosalicylic acid was observed at high temperatures [19]. Scoutaris and his team formulated controlled release dosage form of felodipine using inkjet printing technology and found that the technique was suitable for printing as a high drug loading and desired release characteristics was observed [20]. Moreover, various researches were done by researchers across the world, which showed that the personalized dosage forms containing multiple drugs can be prepared using 3D printing technique with different drug release profile in near future [30].

3.3 Topical devices

The topical devices can easily be fabricated using 3D printing technology with accuracy and precision in drug-loading as per the need of individual patient [4]. Goyanes with his team formulated a nose-shaped mask loaded with drug salicylic acid for the treatment of acne vulgaris using 3D scanning technology. They also compared the suitability and efficiency of stereolithography and fused deposition modeling techniques. It was found that stereolithography showed more efficient results in drug loading and resolution than FDM, and most importantly no degradation of drug was observed. From this study, it was concluded that the 3D printing method efficiently produces tailored drug-loaded devices in required size and shape as per individual's requirement [21]. 3D printing technique also offers the personalized therapy in case of multidrug topical delivery systems with modified release characteristic [31].

3.4 Immediate release tablets

To offer immediate effect of the drug, immediate release drug delivery system is formulated using the 3D printing technique by adding hydrophilic polymer into it. The different polymers like hydroxyl propyl methylcellulose (HPMC), hydroxyl propyl cellulose (HPC), and povidone can be utilized. Okwuosa and team prepared the immediate release tablets of dipyridamole and theophylline by adding PVP as polymer, talc as filler, and triethyl citrate (TEC) as plasticizer in different ratios. The results showed rapid dissolution of more than 90% of the drug with approximately 10% loading [32].

3.5 Pulsatile drug release tablets

The novel pulsatile drug delivery system is developed as 3D printing techniques to produce a new generation design capsules (Chronocaps). Melochhi studied and compared the evaluation parameters of capsular design prepared by both injection molding and 3D printing technique. The results showed lag phase before release, which leads to get longer duration of action in case of 3D printed product [23]. Qijun Li et al. developed controlled-release glipizide novel drug delivery device by combining fused deposition modeling (FDM) with hot melt extrusion (HME). The drug-loaded 3D printed device was successfully developed and evaluated. It was found that the drug release pattern fits Korsmeyer-Peppas release kinetics [33].

3.6 Sustained release tablets

3D-printed sustained release tablets were prepared using fused deposition modeling comprising the drugs as 5-aminosalicylic acid plus 4-aminosalicylic acid.

Polyvinyl alcohol was added in the preparation to get the desired drug loading. The drug loading was found as 0.06% w/w for 5-aminosalicylate and 0.25% w/w for 4-aminosalicylate, respectively. It also showed 100% drug release within 4 hours, making this technique suitable for better therapeutic outcome [34].

3.7 Biphasic release tablets

Khaled with team formulated the 3D-printed bilayer tablet of guaifenesin which was compared with the marketed bi-layer tablet called Mucinex®. The formulated bilayer tablet was made up of sustained release (SR) and immediate release layer (IR). The IR layer comprised API guaifenesin added with binder (HPMC 2910), MCC (microcrystalline cellulose), and sodium starch glycolate (SSG) as disintegrants. While the SR layer was made up of polyacrylic acid and hydroxypropyl methylcellulose (HPMC 2208). It was found that the tablet comprising 2% binder and 14% w/w HPMC 2208 dissolved faster. But, it was also observed that, with an increase in concentration of HPMC 2208, the rate of dissolution got decreased [35].

3.8 Transdermal patches

The transdermal patches offer a multiple layer for getting continuous relief for a prolonged period of time. These patches can be prepared in two separate strategies: first is the reservoir patch where drug along with membrane form a compartment, which control the drug delivery through the system. This design helps to release a drug in a stable kinetics, but defective membrane may release the overdose of medicament. Second, to overcome this drawback, matrix type patch is produced because of the release of the drug into the skin [36].

3.9 Microneedles

These microneedles help to bypass stratum corneum along with the first pass hepatic metabolism [37]. In 2012, Innoture Limited was patented for "Method of preparation of a microneedle or micro implant." The microneedles were formulated by the deposition of a layer onto the surface in a sequential manner and later on deposition of multiple layers one over other to form a solidified needle shaped substance called as micro-implant. This was developed using methods like drop on drop and drop on solid deposition, where a base is formed onto the substrate followed by the deposition of multiple layers to form a microneedle shaft and then its subsequent solidification. The company also developed a microneedle patch known as Radara® to be used as a skincare product, in which hyaluronic serum is delivered at the target site to enhance skin hydration [38].

3.10 Cancer treatment

Many anticancer drugs are unable to reach the specific site of action or even get deposited in essential vital organs and show severe side effect. The oral dosage system and intravenous injection are also unable to give complete therapeutic activity due to poor solubility. Therefore, topical delivery of drug can overcome this problem of conventional delivery system [39]. A patch comprising 5-fluorouracil, poly (lactic-co-glycolic) acid was successfully prepared and implanted on cancer site in pancreas

where the drug was released for about 4 weeks. This system helps to increase patient's acceptability and compatibility.

3.11 Novel dosage forms and drug delivery devices

Various implantable drug delivery devices can be easily prepared with 3D printing technology to get modified drug release profiles. Bone infection and osteoarticular tuberculosis can be immediately treated with such implants [40]. Zhu and co-workers developed an implant for osseous regeneration and to provide a local combinational therapy to minimize side effects and also to avoid drug resistance. They formulated a 3D-printed product with high drug loading of rifampin and isoniazid. They found prolonged release pattern of the drug with maintenance of the desired drug level in target tissues and in blood. This novel technology is used to print various novel dosage forms like nanosuspensions, microcapsules, multilayered drug delivery devices, etc. [41]. Lee et al. prepared paclitaxel microparticles using a piezoelectric inkjet printing with pre-defined and controlled shapes. The technique was found reproducible, accurate, and favorable for bulk production of microparticles. The evaluation was done, and results showed a biphasic release pattern. First, an initial burst followed by slow release. It was also found that the release rate of drug depends onto the geometry of molecule and surface area [42].

Inzana and team used 3D printing technology for the formulation of vancomycin and rifampin-loaded calcium phosphate products to heal the bone infection caused due to implant-associated *Staphylococcus aureus* bone infection [43]. It was resulted that the bacterial metabolic load was reduced with the treatment. Genina and co-workers prepared water impermeable transparent films, oro-dispersible films, and porous copy paper sheets using inkjet printing method. They also evaluated and compared the suitability of these delivery systems. All these systems comprise rasagiline mesylate as a drug. Using thermal inkjet printer, the flexible doses were formulated as a single unit by the deposition of numerous succeeding layers of drug ink onto the top of the previously printed layers. No drug crystals were observed on the surface of the paper sheet after printing due to absorption of the drug ink onto the substrate [44]. The excellent correlation was found between the number of printing layers and the dose in case of porous copy paper than the other two substrates. So, it was finalized that porous copy paper acts as a suitable edible substrate with better absorption properties for the successful formulation of dosage form delivery systems using thermal inkjet printers [45].

3.12 Polypill

Lamichhane S et al. prepared polypill including five compartmentalized drugs using 3D printing technique. The polypill was prepared with distinct release profiles in which two were prepared as a controlled release one [46]. The delivery system prepared for the treatment of cardiovascular complications with an inclusion of hydrochlorothiazide and aspirin as a drug in immediate release compartment, while atenolol, pravastatin, and ramipril were incorporated in sustained release compartments. The formulation was prepared and showed satisfactory results as expected in different release profiles. Further, the same scientist also used extrusion-based 3D printing for the formulation of tablet containing two sustained release compartments for glipizide and nifedipine and another containing an osmotic pump with captopril. From the results, it was found that the captopril showed zero order drug release as expected, while glipizide and nifedipine showed first order release or Korsmeyer-Peppas release kinetics [47].

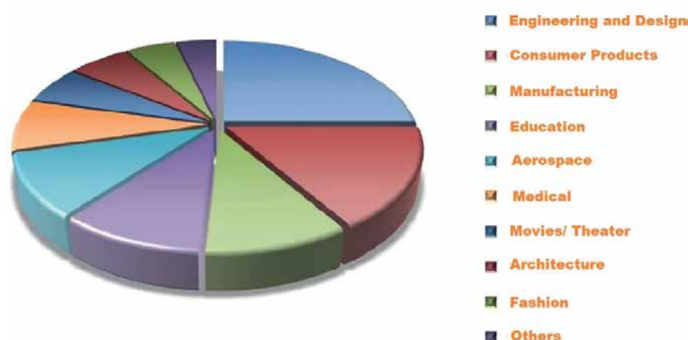

Figure 10.
Application of 3D printing technique.

3.13 On-demand manufacturing

On-demand manufacturing is one of the drug delivery system being useful to fabricate the dose as per patient's needs. It offers immediate printing of drug onto the substrate as per instructions obtained digitally. Such techniques are much useful in emergency cases, where the drugs can be printed especially as per the need of the patient, which expands their utilities during surgery and emergent medicines [30]. On-demand manufacturing is also more applicable in resource-constrained locations like military operations, operating rooms, disaster areas, etc. In 2011, the 3D-printed product of poorly water-soluble drug nitroglycerin was formulated for the treatment of angina pectoris. After 3D printing, the dissolution profile was found to increase (**Figure 10**) [48].

4. Conclusion

The 3D printing technology offers numerous advantages in the pharmaceutical field to formulate the tailored dosage forms as per the patient's needs. Especially the pediatric, geriatric, and dysphagic patients are benefited as such 3D-printed dosage forms can be easily administered showing maximum therapeutic efficacy and minimal side effects. The drugs with poor solubility and incompatibilities and drugs with stability issues can also be fabricated efficiently using 3D printing technology. Moreover, it provides more accuracy and precision in treatment. This technology plays a crucial role in personalized medicine by enabling the customization of drug dosages and formulations to match the unique genetic profiles and medical histories of individual patients. The patient with comorbidities and different phenotypes and genotypes are also being managed with such revolutionary technology. The technique allows incorporation of multiple drugs with complex drug regimen without any incompatibility and also offers targeted drug delivery to the specific site without any alteration of drug concentration. The dosage forms prepared by 3D printing technology is utilized to fabricate the dosage forms by incorporating multiple drugs as per the requirement. Such dosage form is beneficial specially for older population suffering from multiple problems, where single dosage form is administered instead of multiple drugs and also offering prolonged release of drug once administered. The personalized medication is also

helpful for the patients suffering from dementia, dysphagia, etc. This technology can also play a massive role in hospitals and pharmacies, in order to dispense personalized medicines for inpatients. The 3D-printed technology offers rational treatment in case of life-threatening diseases, diagnosis, surgeries, implants, etc. The various 3D printing methods including inkjet printing, stereolithography, laser-based writing systems, and nozzle-based deposition systems are useful to fabricate the drugs into 3D-printed products in a wide range. In future, the approach of 3D printing technology in personalized medicine holds significant potential not only at a commercial level but also for the fabrication of personalized doses in resource-constrained areas, further enhancing patient-specific treatments and improving healthcare outcomes.

Author contribution

Pravin Admane contributed to conceptualization, writing the original draft, and reviewing and editing. Sheetal Mane participated in conceptualization, supervision, and reviewing and editing. Kuldeep Vinchurkar was responsible for formal analysis, supervision, and writing the original draft.

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

3D printing	three-dimensional printing
CIJ	continuous inkjet printing
DoD	drop on demand
DoS	drop on solid deposition
SLA	stereolithography
SLS	selective laser sintering
SSE	semisolid extrusion
FDM	fused diffusion modeling

Author details

Pravin Admane[1]*, Sheetal Mane[2] and Kuldeep Vinchurkar[3]*

1 Gurunanak Technical Institute, Nagpur, Maharashtra, India

2 School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore, Madhya Pradesh, India

3 Krishna School of Pharmacy and Research, Kiran and Pallavi Patel Global University, Vadodara, Gujarat, India

*Address all correspondence to: pravin.admane02@gmail.com
and kuldeepvinchurkar@gmail.com

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References

[1] Trenfield SJ, Awad A, Madla CM, Hatton GB, Firth J, Goyanes A, et al. Shaping the Future: Recent Advances of 3D Printing in Drug Delivery and Healthcare. Expert Opinion on Drug Delivery. Taylor and Francis Group; 2019. pp. 1-16. DOI: 10.1080/17425247.2019.1660318

[2] Kotta S, Nair A, Alsabeelah N. 3D printing Technology in Drug Delivery: Recent Progress and application. Current Pharmaceutical Design. 2018;**24**(42):5039-5048. DOI: 10.2174/1381612825666190218100302

[3] Afsana JV, Haider N, Jain K. 3D printing in personalized drug delivery. Current Pharmaceutical Design. 2018;**24**:5062-5071. DOI: 10.2174/1381612825666190218100343

[4] Norman J, Madurawe RD, Moore CM, Khan MA, Khairuzzaman A. A new chapter in pharmaceutical manufacturing: 3D-printed drug products. Advanced Drug Delivery Reviews. 2017;**108**:39-50. DOI: 10.1016/j.addr.2016.03.001

[5] Downing J. Cycle Pharmaceuticals to Use 3D Printing to Develop 'Orphan Drugs'. Cambridge Independent; 2018

[6] Derby B. Inkjet printing of functional and structural materials: Fluid property requirements, feature stability, and resolution. Annual Review of Materials Research. 2010;**40**:395-414. DOI: 10.1146/annurev-matsci-070909-104502

[7] Goole J, Amighi K. 3D printing in pharmaceutics: A new tool of design customized drug delivery system. International Journal of Pharmaceutics. 2016;**499**:376-394. DOI: 10.1016/j.ijpharm.2015.12.071

[8] Wong KV, Hernandez A. A review of additive manufacturing. ISRN Mechanical Engineering. 2012;**2012**:1-10. DOI: 10.5402/2012/208760

[9] Awada A, Fina F, Goyanes A, Gaisford S, Basit AW. 3D printing: Principles and pharmaceutical applications of selective laser sintering. International Journal of Pharmaceutics. 2020;**586**:119594, 1-14. DOI: 10.1016/j.ijpharm.2020.119594

[10] Panwar A, Tan LP. Current status of bioinks for micro-extrusion-based 3D bioprinting. Molecules. 2016;**21**(6):685. DOI: 10.3390/molecules21060685

[11] Maher PS, Keatch RP, Donnelly K, Mackay RE, Paxton JZ. Construction of 3D biological matrices using rapid prototyping technology. Rapid Prototyping Journal. 2009;**15**(3):204-210. DOI: 10.1108/13552540910960340

[12] Preis M, Oblom H. 3D-printed drugs for children-are we ready yet? AAPS PharmSciTech. 2017;**18**(2):303-308. DOI: 10.1208/s12249-016-0598-9

[13] Pietrzak K, Isreb A, Alhnan MA. A flexible-dose dispenser for immediate and extended release 3D printed tablets. European Journal of Pharmaceutics and Biopharmaceutics. 2015;**96**:380-387. DOI: 10.1016/j.ejpb.2015.08.018

[14] Mohapatra S, Kar R, Biswal P, Bindhani S. Approaches of 3D printing in current drug delivery. Sensors International. 2022;**3**:1-10. DOI: 10.1016/j.sintl.2021.100146

[15] Tagami T, Fukushige K, Ogawa E, Hayashi N, Ozeki T. 3D printing factors important for the fabrication of polyvinylalcohol filament-based tablets. Biological & Pharmaceutical Bulletin. 2017;**40**(3):357-364. DOI: 10.1248/bpb.b16-00744

[16] Mini E, Nobili S. Pharmacogenetics: Implementing personalized medicine. Clinical Cases in Mineral and Bone Metabolism. 2009;**6**(1):17-24. DOI: 10.11138/ccmbm/2009.6.1.017

[17] Wang J, Goyanes A, Gaisford S, Basit AW. Stereolithographic (SLA) 3D printing of oral modified-release dosage forms. International Journal of Pharmaceutics. 2016;**503**(1-2):207-212. DOI: 10.1016/j.ijpharm.2016.02.074

[18] Awad A, Trenfield SJ, Goyanes A, Gaisford S, Basit AW. Reshaping drug development using 3D printing. Drug Discovery Today. 2018;**23**(8):1547-1555. DOI: 10.1016/j.drudis.2018.05.025

[19] Goyanes A, Buanz AB, Hatton GB, Gaisford S, Basit AW. 3D printing of modified-release aminosalicylate (4-ASA and 5-ASA) tablets. European Journal of Pharmaceutics and Biopharmaceutics. 2015;**89**:157-162. DOI: 10.1016/j.ejpb.2014.12.003

[20] Scoutaris N, Alexander MR, Gellert PR, Roberts CJ. Inkjet printing as a novel medicine formulation technique. Journal of Controlled Release. 2011;**156**(2):179-185. DOI: 10.1016/j.jconrel.2011.07.033

[21] Cui M, Pan H, Fang D, Pan W, Li Q, Wen H, et al. Fabrication of high drug loading levetiracetam tablets using three-dimensional printing technology. Journal of Drug Delivery Science and Technology. 2019;**54**:101377. DOI: 10.1016/j.jddst.2019.101377

[22] Okwuosa TC, Stefaniak D, Arafat B, Isreb A, Wan K, Alhnan MA. A lower temperature FDM 3D printing for the manufacture of patient-specific immediate release tablets. Pharmaceutical Research. 2016;**33**(11):2704-2712. DOI: 10.1007/s11095-016-2027-3

[23] Melocchi A, Parietti F, Loreti G, Maroni A, Gazzaniga A, Zema L. 3D printing by fused deposition modeling (FDM) of a swellable/erodible capsular device for oral pulsatile release of drugs. Journal of Drug Delivery Science and Technology. 2015;**30**:360-367. DOI: 10.1016/j.jddst.2015.06.004

[24] Wallis M, Al-Dulimi Z, Luo Q, Tan DK, Yin HF, Arafat B. Feasibility of FDM 3D printing of zein as an excipient for directly compressed pharmaceutical tablets. International Journal of Pharmaceutics. 2020;**587**:119653. DOI: 10.1016/j.ijpharm.2020.119653

[25] Tian P, Wang J, Gao H, Cui D, Wang J, Han J. Fabrication of drug-loaded tablets by extrusion-based 3D printing using a thermosensitive hydrogel material. International Journal of Pharmaceutics. 2019;**568**:118507. DOI: 10.1016/j.ijpharm.2019.118507

[26] Yi HG, Choi YJ, Kang KS, et al. A 3D-printed local drug delivery patch for pancreatic cancer growth suppression. Journal of Controlled Release: Official Journal of the Controlled Release Society. 2016;**238**:231-241. DOI: 10.1016/j.jconrel.2016.08.011

[27] Sadia M, Arafat B, Ahmed W, Forbes RT, Bonner MC, Trenfield SJ, et al. Adaptation of pharmaceutical excipients to FDM 3D printing for the fabrication of patient-tailored immediate release tablets. International Journal of Pharmaceutics. 2018;**544**(2):285-292. DOI: 10.1016/j.ijpharm.2018.03.031

[28] Prasad LK, Smyth H III. 3D printing technologies for drug delivery: A review. Drug Development and Industrial Pharmacy. 2016;**42**(7):1019-1031. DOI: 10.3109/03639045.2015.1120743

[29] Yu DG, Yang XL, Huang WD, Liu J, Wang YG, Xu H. Tablets with material gradients fabricated by three-dimensional printing. Journal of Pharmaceutical Sciences. 2007;**96**(9):2446-2456. DOI: 10.1002/25jps.20994

[30] Khaled SA, Burley JC, Alexander MR, Yang J, Roberts CJ. 3D printing of tablets containing multiple drugs with defined release profiles. International Journal of Pharmaceutics. 2015;**494**(2):643-650. DOI: 10.1016/j.ijpharm.2015.04.02

[31] Jamróz W, Szafraniec J, Kurek M, Jachowicz R. 3D printing in pharmaceutical and medical applications—Recent achievements and challenges. Pharmaceutical Research. 2018;**35**(9):176. DOI: 10.1007/s11095-018-2454-x

[32] Okwuosa TC, Pereira BC, Arafat B, Cieszynska M, Isreb A, Wan KW, et al. Fabricating a shell-core delayed release tablet using dual FDM 3D printing for patient-centred therapy. Pharmaceutical Research. 2017;**34**(2):427-437. DOI: 10.1007/s11095-016-2064-y

[33] Li Q, Guan X, Cui M, Zhu Z, Chen K, Wen H, et al. Preparation and investigation of controlled-release glipizide novel oral device with three-dimensional printing. International Journal of Pharmaceutics. 2017;**525**(1):5-11. DOI: 10.1016/j.ijpharm.2017.04.052

[34] Pereira BC, Isreb A, Forbes RT, Douroumis D. 3D printed tablets (printlets) with braille and moon patterns as accessible dosage forms. International Journal of Pharmaceutics. 2020;**597**:120197. DOI: 10.1016/j.ijpharm.2020.120197

[35] Khaled SA, Burley JC, Alexander MR, Roberts CJ. Desktop 3D printing of controlled release pharmaceutical bilayer tablets. International Journal of Pharmaceutics. 2014;**461**(1-2):105-111. DOI: 10.1016/j. ijpharm.2013.11.021

[36] Economiduo SN, Lamprou DA, Douroumis D. 3D printing application for transdermal drug delivery. International Journal of Pharmaceutics. 2018;**544**(2):415-424. DOI: 10.1016/j.ijpharm.2018.04.031

[37] Kadry H, Al-Hilal TA, Khattab A, Kim YC, Fussell J, Ozhmukhametova EK, et al. Multi-pulse drug release profile from a 3D printed oral dosage form containing polymeric films. International Journal of Pharmaceutics. 2019;**565**:528-536. DOI: 10.1016/j.ijpharm.2019.05.073

[38] Holländer J, Genina N, Jukarainen H, Khajeheian M, Rosling A, Mäkilä E, et al. Three-dimensional printed PCL-based implantable prototypes of medical devices for controlled drug delivery. Journal of Pharmaceutical Sciences. 2016;**105**(9):2665-2676. DOI: 10.1016/j.xphs.2016.04.025

[39] Goyanes A, Det-Amornrat U, Wang J, Basit AW, Gaisford S. 3D scanning and 3D printing as innovative technologies for fabricating personalized topical drug delivery systems. Journal of Controlled Release. 2016;**234**:41-48. DOI: 10.1016/j.45jconrel.2016.05.061

[40] Öblom H, Sjöholm E, Rantanen J, Genina N. 3D-printed isoniazid tablets for the treatment and prevention of tuberculosis-personalized dosing and drug release. AAPS PharmSciTech. 2019;**20**(2):52. DOI: 10.1208/s12249-018-1271-3

[41] Zhu M, Li K, Zhu Y, Zhang J, Ye X. 3D-printed hierarchical scaffold for localized isoniazid/rifampin drug delivery and osteoarticular tuberculosis therapy. Acta Biomaterialia. 2015;**16**:145-155. DOI: 10.1016/j.actbio.2015.01.022

[42] Lee B, Yuna Y, Choi J, Choi Y, Kima J, Choa Y. Fabrication of drug-loaded polymer microparticles with arbitrary geometries using a piezoelectric inkjet printing system, International Journal of Pharmaceutics. 2012;**427**:305-310. DOI: 10.1016/j.ijpharm.2012.02.011

[43] Kates S, Awad H. 3D printed bioceramics for dual antibiotic delivery to treat implant-associated bone infection. HHS Public Access, European Cells and Materials. 2015;**30**:232-247. DOI: 10.22203/ecm.v030a16

[44] Genina N, Jansen EM, Breitenbach A, Breitkreutz J, Sandler N. Evaluation of different substrates for inkjet printing of rasagiline mesylate. European Journal of Pharmaceutics and Biopharmaceutics. 2013;**85**(3 Pt B): 1075-1083. DOI: 10.1016/j.ejpb.2013.05.023

[45] Zhang J, Feng X, Patil H, Tiwari RV, Repka MA. Coupling 3D printing with hot-melt extrusion to produce controlled-release tablets. International Journal of Pharmaceutics. 2017;**519**(1-2):186-197. DOI: 10.1016/j.ijpharm.2016.12.019

[46] Lamichhane S, Sokeng EK, Karki R, Chandrasekaran AR, Elsabahy M, Patil H, et al. Complex multi-layer extrusion-based 3D printing of immediate and sustained-release tablets. International Journal of Pharmaceutics. 2021;**595**:120255. DOI: 10.1016/j.ijpharm.2020.120255

[47] Khaled SA, Burley JC, Alexander MR, Yang J, Roberts CJ. 3D printing of five-in-one dose combination polypill with defined immediate and sustained release profiles. Journal of Controlled Release. 2015;**217**:308-314. DOI: 10.1016/j.jconrel.2015.09.028

[48] Li Q, He X, He J, Xie Y, Han X, Zheng X, et al. Fabrication of sustained-release 3D-printed hydrophilic matrix tablets using a novel water-based extrusion 3D printer. Journal of Pharmaceutical Sciences. 2018;**107**(10):2828-2836. DOI: 10.1016/j.xphs.2018.05.013

Chapter 6

Role of Metabolomics in Precision Medicine in the Context of Systemic Lupus Erythematosus and Lupus Nephritis

Alejandra Rojo-Sánchez, Agustín Abuchaibe, Ada Carmona, Valentina Arrieta-Bravo, David Chica-Valle, Yirys Díaz-Olmos and Lisandro Pacheco-Lugo

Abstract

Systemic lupus erythematosus (SLE) is an autoimmune, multisystemic disease, the underlying causes of which are not fully understood. Clinically, SLE is a heterogeneous disease, and its clinical spectrum overlaps with other rheumatic diseases. Even though multiple organs can be involved in the progression of the disease, the kidney is the main indicator of morbidity and mortality in SLE, a condition known as lupus nephritis (LN). The diagnosis of LN still requires an invasive procedure that becomes impractical when monitoring patients with a confirmed diagnosis, and the described biomarkers do not meet the characteristics of a good biomarker. Recently, "omics" technologies have revolutionized the field of the molecular diagnosis, including autoimmune diseases. Metabolomics has been an extremely useful tool for identifying non-invasive biomarkers in the clinical context and is proposed as a powerful and promising tool to differentiate between individuals with SLE without renal damage and patients with SLE with renal involvement. In addition, this methodology will allow the categorization of patients according to renal damage, promising personalized management of LN in the context of SLE. Here, we review the potential of untargeted metabolomic approaches using LC/GC-MS and MNR to identify potential biomarkers for renal damage in patients with SLE.

Keywords: systemic lupus erythematosus, lupus nephritis, metabolomics, LC-MS, GC-MS, biomarkers

1. Introduction

Systemic lupus erythematosus (SLE) presents a high clinical complexity due to its wide variety of expression patterns. It can affect any organ and follows a relapsing-remitting course, with periods of activity and inactivity. Its multisystemic nature and the wide range of symptoms it can manifest require a careful diagnostic study and the

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use of differentiated therapy based on the severity of the condition [1]. The kidney is affected to varying degrees; in the majority of SLE patients, immune complex deposits are observed, only detectable at present by electron microscopy or immunofluorescence [2]. Renal involvement, or lupus nephritis (LN), can be silent or manifest with clinical patterns known as nephritic or nephrotic syndrome, high blood pressure, and progressive deterioration of kidney function, eventually leading to end-stage renal failure [3]. Approximately 75% of patients with SLE develop renal involvement at some point in the course of the disease, of which 25 to 50% develop nephropathy in the early stages of the condition [4]. The percentages for different types of LN are 25% for focal (class III according to the World Health Organization), 37% for diffuse (class IV), and 13% for membranous (class V) [5].

LN is a frequent and severe manifestation of SLE. The prevalence of LN varies depending on age, gender, ethnicity, and other factors, occurring in approximately 50–60% of SLE patients, of which up to 10% will develop end-stage renal disease [6, 7]. The incidence of SLE varies by region, with higher rates in the United States compared to Europe [8, 9], and by ethnicity, with higher rates in Black individuals (34–51%), followed by Hispanics (31–43%) and Asians (33–55%). There is also a relationship between ethnicity and the severity of symptoms, with Black individuals exhibiting more severe symptoms [10, 11].

The pathogenesis may involve a variety of mechanisms, including gene expression leading to neutrophil activation, increased production of interferon and other proinflammatory myeloid mediators, release of neutrophil extracellular traps (NETs), and complement activation. The glomerular lesion pattern observed in SLE is generally related to the binding of antibodies to multiple intrarenal autoantigens or the formation of circulating antigens, forming immune complexes containing autoantibodies deposited in various parts of the glomerulus, mainly due to antibodies against double-stranded DNA (anti-dsDNA or anti-DNA) [10, 12, 13]. Molecular phenotyping of the kidney or urine can identify the current immunological and/or injury process affecting each individual, leaving a metabolic footprint. This allows for the evaluation of diagnostic and/or monitoring biomarkers for LN and expands knowledge of pathogenesis [14–16].

Renal biopsy remains the gold standard for confirming the diagnosis of LN and determining the histological type and extent of damage, using the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification [17]. Fluid biomarkers, which are validated indicators of physiological or pathological processes or treatment response, are currently considered less invasive alternatives or complementary means of evaluating SLE-related kidney disease [18]. Traditional laboratory biomarkers include immunoserological tests such as anti-double-stranded DNA (dsDNA) levels, complement levels, and parameters associated with renal disease, such as 24-hour urinary protein or urinary protein/creatinine ratio, urine sediment, and glomerular filtration rate (GFR). They are well-established tools for clinical LN evaluation. However, they do not detect LN at early stages of the disease, presenting limited sensitivity and specificity for distinguishing between active disease and chronic lesions [19].

Recent technological advancements, including deep proteomic and metabolic analysis tests, have contributed to the evidence of new biomarkers, some of which have shown performance equivalent to traditional markers [20, 21].

The concept of metabolomics includes the systematic detection and quantification of low-molecular-weight molecules produced by cells (endogenous metabolites) that leave unique chemical traces [15, 22]. Metabonomics, on the other hand, is defined as the quantitative measurement of changes in these metabolic profiles [23]. The theoretical basis lies in the idea that disturbances in a biological system, caused by a

disease, for example, will be detectable as changes in the concentrations of certain metabolites. This also provides the possibility of revealing associated underlying biochemical phenomena, thus offering insights that help in developing a better understanding of disease pathogenesis [15].

In some cases, it may be possible to identify a single robust diagnostic metabolite, as in congenital metabolic diseases. Still, in many cases, the disturbances are more subtle, involving the activation of multiple enzymatic (biochemical) pathways, making it unlikely that a single biomarker will be specific enough for diagnostic purposes [15]. However, by using multivariate statistics, it may be possible to describe changes in biomarker patterns rather than individual biomarkers [15]. Nuclear magnetic resonance spectroscopy (NMR) and liquid and gas chromatography coupled with tandem mass spectrometry (LC-MS/MS and GC-MS/MS) are the most widely used analytical techniques for metabolomic studies due to their great power to identify new metabolites in different biofluids in an unbiased manner. In this book chapter, we will review the literature related to the application of metabolomic techniques for the study of biomarkers in individuals with SLE and LN and the impact these technologies can have on personalized medicine.

2. Role of metabolism disturbances in the context of SLE

2.1 TCA cycle

Within the many possible explanations for the pathogenesis of SLE, it has been established that abnormal T cell activation and cell apoptosis, which are highly energy-dependent processes, play a major role in the progression of the disease. SLE patients exhibit mitochondrial abnormalities that affect energy production and cell survival [24, 25]. A study conducted by Yan et al. that applied gas chromatography–mass spectrometry to analyze serum samples found that SLE patients had lower levels of fumarate and citrate (**Table 1**) [26], which are intermediates of the energy-producing tricarboxylic acid (TCA) cycle. This suggests that SLE disrupts cellular energy metabolism, and the extent of this dysfunction may be related to disease activity. This same study also reported that patients with SLE had lower levels of glucose in the sera compared to healthy controls, attributed to an upregulation of the pentose phosphate pathway in SLE, which requires a higher rate of glucose consumption and a subsequent disturbance of glucose utilization [26, 34].

2.2 Oxidative stress

SLE is characterized not only by abnormal T cell activation but also by an increase in oxidative stress [35, 36], which has been associated to alterations in the immune system and autoantibody production, as well as to the cardiovascular complications of the disease [26, 37]. Glutathione is an important antioxidant that has been found to have significant implications in nutrient metabolism and regulation of cellular events such as gene expression, synthesis of proteins and DNA, cellular proliferation, apoptosis, signal transduction, and protein glutathionylation [38, 39]. By the subsequent actions of glutamate cysteine ligase (GCL) and GSH synthetase, this antioxidant is created from constituent amino acids including cysteine, glutamate, and pyroglutamarate [26, 38]. Glutathione is responsible for suppressing the development of Th17 cells, a subset of T cells involved in autoimmune responses, and for

Metabolite	Type of sample	Disease	Method applied	References
Fumarate, citrate, glucose	Serum	SLE	GC-MS	[26]
Glutathione	Serum	SLE	HPLC	[24]
Aminomalonate, threonate, alpha-tocopherol	Urine	SLE	GC-MS	[14]
L-pyroglutamic acid, adenosine	Serum	SLE	LC-MS-MS	[27]
Adenosine	Feces	SLE	UHPLC-MS-MS	[28]
Arginine	Serum	SLE	LC-MS and GC-MS	[29]
2-Hydroxyisovalerate, 2-keto-3-methylvalerate, and 2-ketoisocaproate	Serum	SLE	GC-MS	[26]
Glutamine	Brain Imaging	SLE	H single-voxel spectroscopy	[30]
Tryptophan, kynurenine	Serum	SLE	UHPLC	[31]
Kynurenine and Kynurenic acid	Peripheral Blood Lymphocytes	SLE	LC-MS-MS	[32]
2-hydroxyisobutyrate, glycerol, oleic acid, arachidonic acid, 1-monopalmitin, and linoleic acid	Serum	SLE	GC-MS	[26]
Deoxycholic acid, glycolic acid, ursodeoxycholic acid, and arachidonic acid	Feces and serum	SLE	LC-MS	[33]

Table 1.
Metabolites in SLE.

reducing intracellular levels of reactive oxygen species (ROS), which are known to contribute to tissue damage [24, 40]. Additionally, glutathione regulates the rise of the mitochondrial transmembrane potential, thereby influencing the activation of the mammalian target of rapamycin (mTOR) pathway in T cells of SLE patients [41, 42]. The dysregulation of the mTOR pathway is connected to the development of SLE and will be further explored later in this chapter.

A study using high-performance liquid chromatography performed by Gergely et al. [24] reported decreased levels of glutathione in the peripheral blood of SLE patients, indicating a deficiency in this important antioxidant (**Table 1**). In an effort to adjust glutathione levels and modulate disease severity, a couple of studies administered N-acetylcysteine (NAC), a cell-permeable precursor of cysteine and a rate-limiting component of *de novo* reduced glutathione synthesis, to both human patients and mouse models of SLE [42, 43]. The administration of NAC resulted in the inhibition of the mTOR pathway in T cells, resulting in a reduction in disease severity and a decrease in organ damage [38, 39, 44]. In general, SLE patients exhibit insufficient levels of both glutathione and GCL activity. In nonpathogenic states, glutathione and GCL play vital roles in protecting against oxidative stress, detoxifying xenobiotics, and maintaining cellular homeostasis. However, in SLE, these crucial components of the antioxidant defense system are compromised [45].

In addition, a study using gas chromatography/mass spectrometry on urine samples revealed that individuals with SLE exhibited reduced levels of aminomalonate,

threonate, and the antioxidant alpha-tocopherol, which are crucial in regulating oxidative stress [14, 46]. Furthermore, metabolic profiling based on liquid chromatography-tandem mass spectrometry detected elevated levels of L-pyroglutamic acid in serum samples from SLE patients, indicating its potential as a disease biomarker [27]. Also known as 5-oxoproline, L-pyroglutamic acid is a derivative of glutamate *via* the γ-glutamyl cycle, a process in which glutathione decomposes into a γ-glutamyl amino acid and is subsequently converted to pyroglutamic acid through γ-glutamyl cyclotransferase [47]. Given the confirmed decrease in serum glutathione in SLE patients [24], it is suggested that the elevated levels of L-pyroglutamic acid in their serum result from changes in glutathione metabolism. Zhang et al. found a positive correlation between the concentrations of L-pyroglutamic acid and the erythrocyte sedimentation rate (ESR) and anti-Sm antibody in SLE patients, indicating its potential as a marker for disease progression [27]. These findings collectively underscore the imbalanced oxidant/antioxidant system in SLE patients compared to healthy controls, as evidenced by multiple studies demonstrating alterations in glutathione metabolism.

2.3 Purine metabolism

Alterations in the metabolism of purines have also been correlated to the pathogenesis of SLE. A study performed by Zhang et al. that utilized liquid chromatography-tandem mass spectrometry revealed that serum adenosine levels were lower in SLE patients compared to healthy individuals [27]. This outcome aligns with a metabolomic investigation involving feces samples from SLE patients [28]. Adenosine, an endogenous purine nucleoside, holds great significance as Treg cells produce it as a potent immunosuppressive agent, thereby reducing immune responses to oneself, regulating tolerance to tissue grafts, and providing protection against autoimmune conditions [48, 49]. Gao et al.'s study [50] also revealed a notable rise in the blood adenosine deaminase activity, an enzyme involved in adenosine hydrolysis, among individuals with SLE, which may account for the reduced blood adenosine levels observed in these patients. The pathophysiology of SLE is frequently characterized by an imbalance between Th17 and Treg cells. This imbalance involves a reduction in Treg cells and an elevation in Th17 cells, which commonly exhibit functional abnormalities [51]. Consequently, the reduction in adenosine could in addition be linked to the imbalance of Treg cells and could be used to predict the population of Treg cells in individuals with SLE [27].

Finally, in relation to purine metabolism, a study investigating the intestinal dysbiosis in individuals with SLE [52] identified a clear association between purine metabolism and the prevalence of the genus *Streptococcus*, which was significantly higher in SLE patients. The known role of adenosine in facilitating intestinal epithelial repair and anti-inflammation has been established [53, 54], suggesting that fecal purine metabolism serves as an informative indicator of the gut microbiome in SLE patients. These results imply that adenosine or purine metabolism, which affects gut flora, inflammation, and systemic immunity, has a role in the pathogenesis of SLE [27, 28].

2.4 Amino acid metabolism

In the context of SLE, amino acids are key nutrients for proliferating T cells as they serve both as a source of energy and as precursors for protein and nucleic acid biosynthesis. Glutaminolysis is essential for the production of Th1 and Th17, pro-inflammatory effectors T cells. The enzymes involved in this pathway are of

great interest and thus have been examined in the context of pathogenic states [38]. Several investigations have also suggested that amino acid transporters are essential components for T cell clonal proliferation and differentiation in response to antigen presentation. Particularly, it has been demonstrated that the loss of the Large Neutral Amino Acid Transporter 1 (LAT-1), also known as the L-system Slc7a5 transporter, which is a transporter devoted to the transport of essential amino acids, inhibits CD4+ and CD8+ T cell differentiation and proliferation but does not impair CD4+ T cell differentiation into regulatory T cells. T cells without Slc7a5 trancarrier do not increase glutamine and glucose uptake and do not change their metabolism to aerobic glycolysis after stimulation of the T cell receptor. This lack of availability of amino acids leads to inadequate mTORC1 activation, which is required for CD4+ cell differentiation into the T-helper (Th1) and Th17 subsets [55, 56].

Branched-chain amino acids (BCAAs) include valine, leucine, and isoleucine. More than half of the body's overall metabolism is accounted for by the metabolism of amino acids in muscle tissue, with skeletal muscle being the primary site of BCAA catabolism [26, 57]. Branched amino acids are known to function as signaling molecules that control the synthesis of proteins, lipids, and glucose [38]. Through unique signaling networks, particularly the phosphoinositide 3-kinase signaling – protein kinase B – mTOR pathway (PI3K/AKT/mTOR), they can also contribute to gut health and immunity [58].

The activity of the target of rapamycin (mTOR) is regulated by several elements such as the availability of amino acids, the energy levels, and growth factors. In mammalian cells, mTOR forms two distinct complexes: the mTORC 1 complex (mTORC1) and the mTORC 2 complex (mTORC2). The mTORC1 complex detects several signs of stress, including the accumulation of amino acids such as leucine, isoleucine, and glutamine. According to studies, Th17 cells and T cells that produce IL-4 have increased mTORC activity, which contributes to the proinflammatory profile seen in SLE patients. Additionally, mTOR is necessary for Th 17 cell development because it promotes the synthesis of hypoxia inducible factor 1α (HIF1α), which enhances inflammatory cells' glycolysis in the pseudo-hypoxia state that is characteristic of subjects with SLE [59].

A study performed on murine models showed that the leucine antagonist, N-acetyl-leucine amide (NALA), can inhibit mTORC1 activity and T cell function by compromising the production of IL-2 and IFγ in Th1 cells [60]. It has been reported that leucine is essential for Treg cell function since it promotes mTORC1 activity in Treg cells through the small G RagA/B and Rheb1/2 proteins, inducing its suppressive activity by boosting the expression of the inducible T cell co-stimulator (ICOS) and CTLA4. According to the study, mice with Rheb1-Rheb2- or RagA-RagB-deficient Treg cells experienced a diminished effector activity of Treg cells and developed an autoimmune illness resembling scurvy [58].

The first steps in the breakdown of BCAAs involve decarboxylation by the branched-chain alpha-keto acid dehydrogenase (BCKDH) complex or transamination by aminotransferases (BCATs). Following these processes, metabolites of BCAAs are changed into succinyl- and acetyl-CoA, which are then involved in the tricarboxylic acid cycle (TCA). BCAT has a negative regulatory effect on glycolysis and mTOR in CD4+ T cells. Activated T cells from mice with cytosolic branched chain aminotransferase (BCATc) deficiency showed an increase in mTORC1 activation compared to control healthy mouse T cells [61]. Furthermore, a different study found that oral delivery of the leucine analog ERG240 specifically suppressed BCAT1 activity, lessening the severity of proliferative glomerulonephritis and collagen-induced arthritis in mice [62].

A GC-MS metabolomics study additionally identified a decrease in the concentrations of BBCAs and their metabolites such as 2-hydroxyisovalerate, 2-keto-3-methylvalerate, and 2-ketoisocaproate, indicating SLE may be involved in increased energy production, gluconeogenesis, and protein synthesis or decreased protein degradation or both [26].

During amino acid catabolism, the carbon skeleton and amino groups are processed in separate but interconnected pathways: the TCA and the urea cycles, respectively. Amino acids entering the TCA cycle contribute to energy generation, but in humans, the oxidative energy derived from amino acid catabolism comprises only a small fraction. Thus far, peripheral blood samples from SLE patients have shown a reduction in most of the amino acids examined, including gluconeogenic and ketogenic amino acids. The amino group's catabolic result is ammonia, which is then transformed into urea *via* the urea cycle and eliminated in the urine. A metabolomic study carried out the measurement of the metabolites related to the urea cycle, finding that arginine, the immediate precursor of urea, and urea were increased in subjects with SLE, suggesting increased urea cycle activity in SLE, compared to healthy individuals [29, 63]. On top of this, a GC-MS metabolomics study identified a decrease in the concentrations of BBCAs and their metabolites such as 2-hydroxyisovalerate, 2-keto-3-methylvalerate, and 2-ketoisocaproate. This suggests that SLE may be associated with increased energy production, gluconeogenesis, protein synthesis, decreased protein degradation, or both [26].

Glutamate appears to be a particularly critical amino acid for T cell activation because, upon their activation, T cells consume glutamine at rates that are comparable to or higher than those of glucose [64]. Through the amino acid transporter 2 (ASCT2), CD4+ T cells are able to bind glutamine, which in turn affects the *in vivo* and *in vitro* production of proinflammatory Th1 and Th17 cells. It has been noted, meanwhile, that neither Th2 nor regulatory T cell immunological responses are impacted by the genetic ablation of ASCT2. ASCT2−/− T cells exhibit a reduction in oxygen consumption, lactate generation, and glucose uptake, indicating that glutamine is crucial for the way T cells respond to abrupt changes in their metabolic needs [37, 65].

Glutamine is also a fundamental component of protein synthesis and has a role in the production of fatty acids, nucleotides, and redox regulation, among other critical activities that promote T cell proliferation. Citrate, which is produced from glycolytic pyruvate, is exported from the mitochondria and employed in the synthesis of lipids in activated lymphocytes. Through the cycle of tricarboxylic acids, α-ketoglutarate, which is produced from glutamine, contributes to the formation of citrate. After that, citrate is used to produce acetyl groups for fatty acid synthesis. By means of this process, α-ketoglutarate supplies the building blocks needed to synthesize polyamines, which are essential for the synthesis of nucleotides. Ultimately, glutamate—the initial byproduct of glutamine oxidation—acts as a metabolic bridge for the production of glutathione and has a significant impact on lymphocytes' state of oxidative stress [37, 56].

Glutaminase, the enzyme that is responsible for converting glutamine into glutamate, promotes the proliferation and activation of Th17 cells through various mechanisms. The cAMP responsive element modulator, which is overexpressed in T cells of SLE patients and MRL/lpr mice prone to SLE, regulates the production of glutaminase [66]. Multiple investigations have been carried out to elucidate the impact of this enzyme on the pathophysiology of SLE. Bis-2-(5-phenylacetamide-1,3,4-thiadiazole-2-yl sulfide) ethyl (BPTES), a glutaminase 1 hybridizer, has been shown to reduce Th1 cell development and disease activity in mice with experimental autoimmune

encephalomyelitis [67]. This inhibitor, BPTES, also improves disease activity SLE in MRL/lpr mice [55]. Lastly, another enzyme, glutamate oxaloacetate transaminase 1 (GOT1), which converts glutamate to α-ketoglutarate, helps to potentiate Th17 differentiation through epigenetic processes. Selective inhibition of GOT1 by small hairpin (shRNA) RNA silencing or aminooxyacetic acid (AOA) therapy decreased notably the Th17 differentiation of murine T cells [55].

Furthermore, a study based on H single-voxel spectroscopy specifically identified glutamine as a potential biomarker for cerebral disease activity in SLE patients since the levels of glutamine were found to be altered in the brain of SLE patients even before neurologic and imaging manifestations emerged [26, 30].

Another metabolite that has been associated with SLE pathogenesis is tryptophan. This is an essential amino acid whose bioavailability is strictly based on dietary supplement and the subsequent degradation by the intestinal microbiota [43]. Tryptophan is the precursor of several metabolites produced by microbial and endogenous enzymes. The tryptophanase enzymes (Tnase) in the gut microbiota convert dietary tryptophan into bioactive catabolites, including indole and other indole derivatives *via* several pathways. Moreover, tryptamine, which is produced by some bacteria *via* the enzyme tryptophan decarboxylase, controls intestinal transit by binding to serotonin receptors [68].

Multiple mechanisms have been proposed to explain how tryptophan contributes to the development and progression of lupus. Low tryptophan concentrations but high kynurenine levels in the serum of lupus-prone mice were indicative of a skewed tryptophan metabolism through the kynurenine pathway, as reported by Brown et al. [31]. In addition, Pearl et al. [32] showed, thanks to a quantitative metabolome analysis based on peripheral blood lymphocytes, that N-acetylcysteine (NAC) therapy may be able to reverse the accumulation of kynurenine and kynurenic acid in patients with SLE. Exogenous kynurenine has been shown in some studies to enhance Th1 polarization of CD4+ T cells and decrease Treg cell polarization of cytotoxic T cells, indicating that kynurenine supports pro-inflammatory T cell phenotypes. According to the same study [32], kynurenine causes the activation of the target of rapamycin (mTOR) in human T cells, which contributes to the high level of mTOR activation that characterizes CD4+ T cells in patients with SLE. Proinflammatory cytokines, such as interferon gamma (IFNγ) and interleukin 17 (IL-17), which are essential to the pathophysiology of SLE, have also been observed to be over-expressed and over-activated in CD4+ T cells with active hypomethylation of genes in the mTOR pathway [69].

Lastly, many tryptophan-derived metabolites, including indole-3-aldehyde, indole-3-acetic acid, 3-methylindole, and tryptamine, are aryl hydrocarbon receptor (AhR) ligands [31, 70]. Through the regulation of P53, FasR, Bcl-2, and cell cycle kinases, AhR activation controls numerous essential biological functions, including cell cycle progression, apoptosis, and cell proliferation. AhR stimulation increases the regulation of genes encoding cytokines such as IL-10, which regulate immune tolerance in SLE [31].

2.5 Gut-microbiome-derived metabolism

Lupus patients often experience gastrointestinal symptoms that are atypical and nonspecific. Until 2014, rheumatologists and researchers dismissed the idea that gut dysbiosis could be associated with SLE due to the lack of clear evidence. However, a breakthrough came in 2014 when Hevia et al. [71] demonstrated, for the first time in a group of 20 SLE patients, a distinct dysbiosis characterized by a higher abundance

of *Bacteroidetes* and a significant decrease in the *Firmicutes/Bacteroidetes* ratio compared to healthy individuals. Since then, subsequent studies have revealed similarities in SLE-related gut microbiota across different regions, such as Spain and China. In these populations, SLE patients exhibited an enrichment of *Prevotellaceae* compared to their healthy counterparts [70, 72]. Moreover, He et al. identified an increased prevalence of specific genera, including *Rhodococcus, Eggerthella, Klebsiella, Prevotella, Eubacterium, Flavonifractor*, and *Incertae sedis*, along with a decrease in Dialister and Pseudobutyrivibrio [72]. A study by Azzouz et al. [73] found that SLE patients had reduced species richness diversity, particularly pronounced in those with a high SLE Disease Activity Index (SLEDAI). This study highlighted a greater representation of *Ruminococcus gnavus* (RG) of the *Lachnospiraceae* family in SLE patients, which is known to contribute to gut barrier impairment. The combination of increased intestinal barrier permeability and gut dysbiosis in SLE patients has been linked to the translocation of pathogenic bacteria, bacterial endotoxins, toxic metabolites, and an elevation in circulating microbial components. Although these changes may not manifest clinically as infections, they contribute to pathological alterations and immune system dysregulation [74].

The gut microbiota constitutes an intricate assembly of bacteria crucial for digestion, playing a key role in facilitating the breakdown of nutrients through enzymes absent in the human genome [75]. Consequently, bacterial metabolites significantly influence the metabolic processes related to nutrient absorption [70]. Numerous studies have substantiated this concept by delineating the abnormal metabolism of amino acids, lipids, and carbohydrates observed in individuals with SLE [76–78].

A metabolomic investigation using GC-MS revealed a notable elevation of 2-hydroxyisobutyrate in SLE patients experiencing active disease compared to both healthy individuals and SLE patients with inactive disease. This finding suggests a potential link between altered gut microbial metabolism and SLE morbidity, particularly in cases of active SLE, as 2-hydroxyisobutyrate is primarily derived from the degradation of valine by gut microbes [26]. In an ongoing study by our group, we also found a group of bacterial metabolites associated to SLE by urinary metabolomic profiling using LC/GC-MS-MS (unpublished data).

2.6 Lipid metabolism

Examinations of lipid metabolism, conducted in both the serum and feces of SLE patients, indicate a heightened susceptibility to developing lipid profile disorders compared to individuals without the disease [33, 79]. The proposition that dyslipidemia plays a role in the pathogenesis of SLE is substantiated by the elevated occurrence of ischemic heart disease among SLE patients, ranging from 3.8–16%, a figure nearly 10 times greater than that observed in the general population and 50 times higher than the prevalence seen in young women of reproductive age. Furthermore, it has been established that dyslipidemia is prevalent in 68–100% of adult individuals with SLE [80]. Studies exploring lipid metabolism and its association with SLE revealed that more than 60% of altered metabolites in the serum of SLE patients are lipids [33].

Metabolomic and lipidomic investigations have revealed elevated levels of medium-chain fatty acids (MCFA) and free fatty acids (FFA), along with a reduction in long-chain fatty acids (LCFA) in the profiles of individuals with SLE. Short-chain fatty acids (SCFA) can efficiently enter the mitochondrial matrix for beta oxidation directly through the inner mitochondrial membrane, whereas LCFA require assistance from carnitine transport, a process found to be diminished in SLE patients compared to healthy controls [63, 80, 81]. Regarding lipid

membranes, a decrease in most phospholipids was observed in individuals with SLE, possibly indicating an increase in cellular turnover. Lipid metabolism in SLE has also been linked to heightened oxidative stress, evidenced by elevated levels of lipid peroxidation products such as malonaldehyde acid (MDA), 9-hydroxyctadecadienoic acid (9-HODE), and 13-HODE, coupled with a decrease in antioxidants such as α-tocopherol, glutathione, and their precursors. Lipid peroxidation, associated with increased free radical activity and damage to cellular membranes, organelles, and/or DNA, has been implicated in cardiovascular and renal complications in individuals with SLE [82–84].

In an investigation conducted by Yan et al. it was observed that individuals with SLE had notably lower levels of glycerol, oleic acid, and arachidonic acid, while 1-monopalmitin levels were significantly higher compared to control subjects. Glycerol is an essential constituent of triglycerides and phospholipids, whereas 1-monopalmitin falls under the category of monoacylglycerols. In addition, this study revealed that patients with active SLE exhibited lower levels of linoleic acid in comparison to the control group [26].

The impact of lipid metabolism on the functions of immune cells and the pro-inflammatory processes in SLE has been extensively documented. Cells of the innate immune system, like macrophages, engage with oxidized Low-Density Lipoprotein (LDL) particles through scavenger receptors in atherosclerotic plaques. This interaction results in lipid saturation, the production of pro-inflammatory cytokines, and the recruitment of immune cells. In SLE, this process is exacerbated due to elevated circulating levels of LDL and alterations in macrophage function. This dysfunction is induced by the direct activation of hepatic X receptors (LXRs) by lipids, which regulate cellular cholesterol levels and immune functions, influencing factors such as the production of IL-23 and IL-17 and phagocytic pathways [85, 86].

The hyperlipidic environment in individuals with SLE has also been reported to impact T cells. Elevated levels of oxidized LDL have been shown to indirectly enhance T cell activation through monocyte uptake [85, 87]. Furthermore, SLE patients exhibit increased cholesterol and glycosphingolipids in the membranes of T cells, leading to alterations in the composition of signaling platforms known as lipid rafts. These rafts are crucial for T cell receptors to provide stimulatory signals that regulate cell function and inflammation. This alteration is partly attributed to the expression of genes responsible for lipid metabolism in individuals with SLE, but it could also result from changes in the cellular uptake of LDL/VLDL cholesterol and modifications in cholesterol efflux to High-Density Lipoprotein (HDL) [85, 88].

A combined study utilizing 16S sequencing and LC-MS metabolomics characterized alterations in the gut microbiome, as well as fecal and serum metabolomes in SLE patients. The findings indicated a significant correlation between specific bile acids, including deoxycholic acid, glycolic acid, ursodeoxycholic acid, and arachidonic acid, and the SLE Disease Activity Index (SLEDAI) score in patients. Moreover, these bile acids exhibited a strong predictive ability for disease activity [33]. Besides their well-known role in lipid metabolism, bile acids are also signaling molecules that exercise their functions through the activation of the bile acid receptors. A study reported a decrease in FXR receptors in subjects with SLE and in MRL/lpr models of lupus with liver dysfunction [89]. Notably, the administration of chenodeoxycholic acid, an FXR receptor agonist, in mice demonstrated suppression in the expression of inflammatory cytokines such as TNF-α, IFN-γ, and IL-6.

In addition to their impact on the liver, bile acids were identified to modulate intestinal immunity in a separate study. This research revealed that certain metabolites

derived from lithocholic acid (LCA), such as 3-oxoLCA and isoalloLCA, could inhibit the differentiation of Th17 cells by directly binding to a crucial transcription factor, retinoid-related orphan receptor-γt (RORγt). These metabolites were found to enhance FOXP3 gene expression by generating mitochondrial reactive oxygen species (mitoROS), leading to the expansion of regulatory T cells [70, 90].

3. Metabolomics in urine and its alteration in the context of LN

One of the studies in LN metabolomics was conducted by Kalantari et al., who used 1H nuclear magnetic resonance (NMR-1H) to compare the urinary metabolic profile of LN patients, patients with SLE without renal damage, and healthy controls (HC). In the aforementioned study, biomarkers were identified to assess their diagnostic effectiveness in search of operative characteristics of the receptor (ROC). Three metabolites were used including 3,4-dihydroxyphenylacetdehyde, beta-alanine, and 2,2-dimethylsuccinic acid of which the combination of 2,2-dimethylsuccinic and DOPAL was associated with greater diagnostic sensitivity when determining acute renal injury, suggested as a diagnostic panel for LN with an AUC of 0.89, sensitivity of 81%, and specificity of 100% [91].

Among the pathways impaired in LN pathogenesis, twenty-four pathways were significant. Nicotinate and nicotinamide metabolism, AXIN degradation, and DNA nucleotide excision repair pathways were the top three impaired pathways in LN patients [91]. Souliotis and colleagues also reported DNA repair issues in active LN patients, resulting in higher levels of DNA damage than those with inactive disease [92].

In another study based on gas chromatography/mass spectrometry in patients with SLE and LN, 81 metabolites were detected, of which mainly highlights amino acids, nucleotides, organic acids, amines, fatty acids, glyceric acid, creatinine, p-cresol, myoinositol, treonate, hypurate, and urate [22]. However, this study did not differentiate between patients with SLE and LN.

B-alanine is one of the two amino acids (along with histidine) that produce carnosine. The synthesis and metabolism of carnosine develops mainly at the renal level. Its metabolism favors eliminating reactive oxygen species. A significant decrease in urinary excretion of beta-alanine has been observed in LN patients, which could lead to a decrease in the carnosine content of the kidneys in LN patients, making their kidneys susceptible to oxidative stress insults [91].

2,2-Dimethylsuccinate (2,2-DMS) performs a plasma and urinary function by selectively inhibiting sodium-dependent dicarboxylate transporters (NaDC-3 and NaDC-1), which are located at the level of the proximal renal tubule in the luminal and basolateral plasma membranes. Transporters import intermediates from the Krebs cycle for the cells of the proximal tubule to use as energy substrates, among these compounds is succinate. The import of 2,2-DMS is done with higher speed compared to the suction given at greater affinity with the conveyor. Therefore, a decrease in 2,2-DMS levels in the urine of patients with LN may be secondary to increased activity of these transporters in patients with LN [91].

Class IV of the LN favors the proliferation of endocapillary and extracapillary cells, which would explain the energy requirement. There is also evidence that increased levels of nicotinate and nicotinamide (NMN), which participate in the metabolism of vitamin B3, is directly related to energy metabolism. When increasing dicarboxylate transporters, there is a decrease in levels of 2,2-DMS [91].

3.1 Lipids

Within the sphingolipid family are glycosphingolipids that favor cell proliferation and inflammation. At the renal level, they are related to a variety of diseases including glomerulonephritis, renal neoplasia, and renal polycystosis [93]. Tamara and colleagues aimed to identify the role of glycosphingolipids in LN. They utilized the LMR/MpJ-FasLpr/J (LMR/lpr) mouse model of LN, as well as human renal biopsies and urine samples [93]. Elevations of renal and urinary glycosphingolipids occurred in mice prone to lupus; a relationship was determined with the positive transcriptional regulation of neuraminidase that initiates its function in early stages of the disease and progressively increases its activity favoring the elevation of glycosphingolipids. Urinary levels of neuraminidase and lactosilceramide are higher in patients with lupus and LN. Complex glycosphingolipids are formed with the participation of lactosilceramide, example of which is the formation of gangliosides with the addition of sialic acid residues in conjunction with lactosilceramide. Glycosphingolipids are transported within the cell and are mainly located in the plasma membrane. Neuraminidases are enzymes that eliminate sialic acids and proteins and are located in various cell structures at the level of the lysosome, cytosol, plasma membrane, and mitochondria (NEU 1, 2, 3, 4, respectively), of which NEU1 has the ability to secrete itself extracellularly to act on the plasma membrane. Given the above, there is a regeneration of lactosilceramide through NEU enzymes. These data strongly suggest that glycosphingolipids may serve as early markers of LN [93].

With the evolution of the disease, the histological characteristics of the LN are changing; in the follow-up, good or poor response to treatment is evidenced. However, at the time, it is not possible to determine changes in the LN dynamically and in real time, and it may affect the start of therapy and follow-up and thus the prognosis [23].

In the evaluation of the patient, the differentiation of proliferative LN (Class III/IV) and pure membranous LN (Class V) is complex, since they present signs and symptoms in common; both are associated with proteinuria, alterations in blood pressure and kidney function. Pronounced proteinuria is the hallmark of focal segmental glomerulosclerosis (FSGS). One histological characteristic of FSGS is podocyte injury, leading to varying degrees of proteinuria and potentially hypoalbuminemia. These clinical and histological characteristics can also occur with active LN [23].

Romick et al. conducted a study with the aim of identifying urinary metabolites that could discriminate between proliferative LN (Class III/IV), pure membranous LN (Class V), and primary FSGS, using metabolomics based on NMR spectroscopy [23]. It was determined that the urinary metabolites citrate and taurine allow differentiating between proliferative LN and pure membranous LN (**Table 2**). In Class V, citrate levels are up to eight times lower and taurine levels normal, compared to Class III/IV where citrate levels may be normal and taurine up to 10 times lower. Additionally, the levels of urinary hypurate were evaluated, which allowed us to distinguish with precision between Class V that presents normal levels of taurine, compared with FSGS, with the absence of hypurate [23]. In a study conducted by Ganguly et al., it was demonstrated that LN patients exhibit significantly low levels of urinary creatinine/citrate compared to clinically healthy individuals, levels that increased significantly after 6 months of cyclophosphamide treatment. In the same study, it was shown that LN patients had significantly high levels of the acetate/creatinine ratio in urine, levels that did not change after immunosuppressive therapy [95].

Metabolite	Type of sample	Disease	Method applied	References
3,4-Dihydroxyphenylacetdehyde, beta-alanine 2,2-dimethylsuccinic acid	Urine	LN	NMR-1H	[91]
Neuraminidase, glucosylceramide (GlcCer) and lactosylceramide (LacCer), Neu1	Urine human renal biopsies LMR/MpJ-FasLpr/J (LMR/lpr) mouse model of LN	LN	Matrix-assisted laser desorption/ionization imaging MS (MALDI-IMS)	[93]
Citrate and taurine hypurate	Urine	LN	NMR spectroscopy	[23]
LDL/VLDL lipoproteins, sorbitol, glycolic acid acetate, cortisol, creatinine, L-aspartyl-L-phenylalanine	Serum	LN	UPLC-HRMS	[94]

Table 2.
Metabolites in SLE and LN.

The relationship of the metabolites citrate and taurine with kidney function has been determined. Normally, the kidney filters and reabsorbs metabolites, which allows a metabolic balance, so in case of functional deterioration, an alteration in the metabolic profile will be observed [96].

3.2 Serum metabolomics

In LN, a wide range of alterations in metabolic pathways are observed, including glycolysis, amino acid metabolism, and lipid metabolism, resulting in a consequential elevation or reduction that generates a metabolic signature allowing the distinction of patients with SLE. However, variations are observed depending on the region, population, and disease state. In general terms, patients with LN exhibit elevated serum levels of lipid metabolites (including LDL/VLDL lipoproteins), sorbitol, and glycolic acid metabolites and reduced levels of acetate, cortisol, creatinine, and L-aspartyl-L-phenylalanine [94]. Compared to SLE, metabolic variations are observed, such as an increase in LDL/VLDL lipoproteins (triglycerides and fatty acids) and reduced serum levels of acetate [97].

In lupus nephritis, reduced levels of several glucogenic amino acids (such as glycine, alanine, valine, glutamate, proline, and histidine) and ketogenic amino acids (such as leucine) have been determined. This could lead to abnormal catabolism of amino acids and biosynthesis of important proteins in various biological processes, such as cell cycle advancement, genetic transcription, inflammatory responses, and autoimmunity. It has been described that lower serum histidine levels could be closely related to protein waste, energy, inflammation, and oxidative stress. In general, elevated serum glucose levels and reduced levels of most amino acids guide changes in energy production, such as reduced aerobic glycolysis and the use of metabolites as amino acids and ketone bodies as a source of energy [98, 99].

Li J, Xie X et al. conducted a metabolomic study on human serum that revealed a wide range of differential metabolic signatures in patients with LN and idiopathic nephrotic syndrome (INS). Significant metabolic alterations were found in five metabolites, including cortisol, creatinine, sorbitol, L-aspartyl-L-phenylalanine,

and glycolic acid [94]. Furthermore, combined forms of biomarkers proved to be more effective in the diagnosis of LN than a single one.

The AUC (area under the curve – ROC) was 0.85, the highest obtained, when combining theophylline, oxidized glutathione, and capric acid, which indicates a very good diagnostic accuracy, with a sensitivity and specificity of 87.50 and 67.86%, respectively; according to the study data in general, the positive predictive value (PPV) and the predictive value negative (NPV) were 75.68 and 82.61%, respectively [94].

3.3 Cortisol

Low serum levels of cortisol play a proinflammatory role, being negatively correlated with the degree of systemic inflammation. Zietz et al. reported that serum cortisol is negatively correlated with the degree of systemic inflammation in SLE. The study by Li J, Xie X et al. indicated that a significant decrease in the amount of cortisol is associated with a high risk of LN and perhaps an impact on the patient's prognosis [94].

Straub et al., in their study, related that a low level of serum cortisol may be due to a reduction in adrenal steroidogenesis secondary to the inflammatory process and adrenal insufficiency [97].

On the other hand, in the work carried out by Judd et al., it was concluded that proinflammatory cytokines (such as tumor necrosis factor (TNF)) repress relevant enzymatic steps in steroidogenesis that occurs in adrenalocortical cells. The decrease in cortisol levels in patients with LN may be due to inflammation and adrenal insufficiency in patients with LN [94, 97].

3.4 Glycolic acid

Glycolic acid, or glycocholic acid, is a crystalline bile acid that plays a role in the emulsification of fats. It is an acyl glycine produced during the enzymatic metabolism of bile acids in the colonic environment. This compound acts as a fat solubilizer, facilitating its absorption. Additionally, it contributes to the homeostasis of bile acids, which act as signaling molecules with endocrine functions, thereby promoting the homeostasis of triglycerides, cholesterol, and glucose. In a study, the level of glycolic acid was reduced in patients with LN. The decrease in the level of glycolic acid in plasma could be due to the reduced concentration of glycolic acid in the plasma of LN patients [94].

4. Conclusion

The pathogenesis of SLE and LN is of complex etiology. There are no sufficiently specific biomarkers that allow for the noninvasive or minimally invasive prediction of renal involvement in the context of SLE. With the advent of "omics" approaches, a series of metabolites, amino acids, lipids, and proteins, among others have been discovered that could potentially serve as biomarkers for SLE and LN in the future. Metabolomics has unique characteristics that make it a methodology with enormous potential to discover metabolites associated with imbalances in crucial metabolic pathways for the organism. Large-scale validation of these metabolites discovered by these non-targeted approaches in patient cohorts from different regions will be necessary to uncover their role and impact in personalized medicine.

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Conflict of interests

The authors declare no conflict of interests.

Abbreviations

2,2-DMS	2,2-dimethylsuccinate
9-HODE	9-hydroxyoctadecadienoic acid
13-HODE	13-hydroxyoctadecadienoic acid
AhR	aryl hydrocarbon receptor
Anti-DNA	antibodies against DNA
Anti-dsDNA	antibodies against double-stranded DNA
Anti-Sm Antibody	anti-smith antibody
AOA	aminooxyacetic
ASCT2	alanine serine cysteine transporter 2
AUC	area under the curve - ROC
BCAAs	branched-chain amino acids
BCAT1	branched-chain amino acid transaminase 1
BCATs	branched-chain amino acid aminotransferases
BCKDH	branched-chain alpha-keto acid dehydrogenase
Bcl-2	B-cell lymphoma 2
BPTES	bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide
cAMP	cyclic adenosine monophosphate
CTLA4	cytotoxic T-lymphocyte-associated antigen 4
ERG240	leucine analogue
ESR	erythrocyte sedimentation rate
FasR	Fas receptor
FFA	free fatty acids
FOXP3	transcription factor forkhead box protein 3
FSGS	focal segmental glomerulosclerosis
FXR receptors	farsenoid X receptors
GC-MS-MS	gas chromatography with tandem mass spectrometry
GC-MS	gas chromatography - mass spectrometry
GCL	glutamate cysteine ligase

GFR	glomerular filtration rate
GOT1	glutamate oxaloacetate transaminase 1
GSH	glutathione
HC	healthy controls
HDL	high-density lipoprotein
HIF1α	hypoxia inducible factor 1 subunit alpha
HPLC	high-performance liquid chromatography
ICOS	inducible t cell co-stimulator
IFγ	interferon gamma
IL-2	interleukin 2
IL-4	interleukin 4
IL-6	interleukin 6
IL-10	interleukin 10
IL-17	interleukin 17
IL-23	interleukin 23
INS	idiopathic nephrotic syndrome
ISN/RPS	International Society of Nephrology/Renal Pathology Society
LacCer	lactosilceramide
LAT-1	large neutral amino acid transporter 1
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LCA	lithocholic acid
LCFA	long-chain fatty acids
LDL	low-density lipoprotein
LMR/lpr	LMR/MpJ-FasLpr/J
LN	lupus nephritis
LXRs	liver X receptors
MCFA	medium-chain fatty acids
MDA	malonaldehyde acid
mitoROS	mitochondrial reactive oxygen species
MRL/lpr mice	mice are homozygous for the lymphoproliferation spontaneous mutation
mTOR	mammalian target of rapamycin
mTORC 1	mammalian target of rapamycin complex 1
mTORC 2	mammalian target of rapamycin complex 2
NAC	N-acetylcysteine
NaDC	sodium-dependent dicarboxylate transporters
NALA	N-acetyl-leucine Amide
NETs	neutrophil extracellular traps
NEU1	neuraminidase 1 lysosome
NEU2	neuraminidase 2 cytosol
NEU3	neuraminidase 3 plasma membrane
NEU4	neuraminidase 4 mitochondria
NMN	nicotinamide
NMR-1H	1H nuclear magnetic resonance
NMR	nuclear magnetic resonance spectroscopy
NPV	negative predictive value
p53	tumor protein p53
PI3K/AKT/mTOR pathway	phosphoinositide 3-kinase signaling - protein kinase B - mTOR pathway

PPV	positive predictive value
Rag A/B	Ras-related GTP-binding protein A/B
RG	ruminococcus gnavus
Rheb1/2	Ras homolog enriched in brain protein 1/2
ROC	operative characteristics of the receptor
RORγt	retineic-acid-receptor-related orphan nuclear receptor gamma
ROS	reactive oxygen species
SCFA	short-chain fatty acids
shRNA	short hairpin RNA
Slc7a5 transporter	large neutral amino acid transporter 1 (LAT-1)
SLE	systemic lupus erythematous
SLEDAI	systemic lupus erythematous disease activity index
TCA	tricarboxylic acid
Th1 cells	type 1 T helper cells
Th2 cells	type 2 T helper cells
Th17 cells	type 17 T helper cells
Tnase	tryptophanase enzymes
TNF-α	tumor necrosis factor alpha
TNF	tumor necrosis factor
Treg cells	regulatory T cells
UHPLC-MS/MS	ultra-high performance liquid chromatography tandem mass spectrometry
VLDL	very low-density lipoprotein

Author details

Alejandra Rojo-Sánchez[1], Agustín Abuchaibe[1], Ada Carmona[1], Valentina Arrieta-Bravo[1], David Chica-Valle[1], Yirys Díaz-Olmos[2] and Lisandro Pacheco-Lugo[1*]

1 Facultad de Ciencias Básicas y Biomédicas, Centro de Investigaciones en Ciencias de la Vida (CICV), Universidad Simón Bolívar, Barranquilla, Colombia

2 División Ciencias de la Salud, Universidad del Norte, Barranquilla, Colombia

*Address all correspondence to: lisandro.pacheco@unisimon.edu.co

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References

[1] Ruiz-Irastorza G, Khamashta MA, Castellino G, Hughes GR. Systemic lupus erythematosus. The Lancet Lond Engl. 2001;**357**(9261):1027-1032. DOI: 10.1016/S0140-6736(00)04239-2

[2] Austin HA. Clinical evaluation and monitoring of lupus kidney disease. Lupus. 1998;**7**(9):618-621. DOI: 10.1191/096120398678920749

[3] Cameron JS. Lupus nephritis. Journal of the American Society of Nephrology JASN. 1999;**10**(2):413-424. DOI: 10.1681/ASN.V102413

[4] Hricik DE, Chung-Park M, Sedor JR. Glomerulonephritis. The New England Journal of Medicine. 1998;**339**(13):888-899. DOI: 10.1056/NEJM199809243391306

[5] Golbus J, McCune WJ. Lupus nephritis. Classification, prognosis, immunopathogenesis, and treatment. Rheumatic Diseases Clinics of North America. 1994;**20**(1):213-242

[6] Anders HJ, Saxena R, Hui ZM, Parodis I, Salmon JE, Mohan C. Lupus nephritis. Nature Reviews Disease Primers. 2020;**6**(1):7. DOI: 10.1038/s41572-019-0141-9

[7] Almaani S, Meara A, Rovin BH. Update on lupus nephritis. Clinical Journal of the American Society of Nephrology. 2017;**12**(5):825-835. DOI: 10.2215/CJN.05780616

[8] Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Morbidity and mortality in systemic lupus erythematosus during a 10-year period: A comparison of early and late manifestations in a cohort of 1,000 patients. Medicine (Baltimore). 2003;**82**(5):299-308. DOI: 10.1097/01.md.0000091181.93122.55

[9] Cervera R, Khamashta MA, Font J, Sebastiani GD, Gil A, Lavilla P, et al. Systemic lupus erythematosus: Clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European working party on systemic lupus erythematosus. Medicine (Baltimore). 1993;**72**(2):113-124

[10] Parikh SV, Almaani S, Brodsky S, Rovin BH. Update on lupus nephritis: Core curriculum 2020. American Journal of Kidney Diseases. 2020;**76**(2):265-281. DOI: 10.1053/j.ajkd.2019.10.017

[11] Lim SS, Bayakly AR, Helmick CG, Gordon C, Easley KA, Drenkard C. The incidence and prevalence of systemic lupus erythematosus, 2002-2004: The Georgia lupus registry: The Georgia lupus registry. Arthritis & Rhematology. 2014;**66**(2):357-368. DOI: 10.1002/art.38239

[12] Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, Edens M, et al. Personalized immunomonitoring uncovers molecular networks that stratify lupus patients. Cell. 2016;**165**(3):551-565. DOI: 10.1016/j.cell.2016.03.008

[13] Lech M, Anders HJ. The pathogenesis of lupus nephritis. Journal of the American Society of Nephrology. 2013;**24**(9):1357-1366. DOI: 10.1681/ASN.2013010026

[14] Yan B, Huang J, Dong F, Yang L, Huang C, Gao M, et al. Urinary metabolomic study of systemic lupus erythematosus based on gas chromatography/mass spectrometry. Biomed Chromatogr BMC.

2016;**30**(11):1877-1881. DOI: 10.1002/bmc.3734

[15] Bengtsson AA, Trygg J, Wuttge DM, Sturfelt G, Theander E, Donten M, et al. Metabolic profiling of systemic lupus erythematosus and comparison with primary Sjögren's syndrome and systemic sclerosis. PLoS One. 2016;**11**(7):e0159384. DOI: 10.1371/journal.pone.0159384

[16] Davidson A. What is damaging the kidney in lupus nephritis? Nature Reviews Rheumatology. 2016;**12**(3):143-153. DOI: 10.1038/nrrheum.2015.159

[17] Fanouriakis A, Kostopoulou M, Cheema K, Anders HJ, Aringer M, Bajema I, et al. 2019 Update of the joint European league against rheumatism and European renal association–European dialysis and transplant association (EULAR/ERA–EDTA) recommendations for the management of lupus nephritis. Annals of the Rheumatic Diseases 2020;79(6):713-723. DOI: 10.1136/annrheumdis-2020-216924

[18] Califf RM. Biomarker definitions and their applications. Experimental Biology and Medicine. 2018;**243**(3):213-221. DOI: 10.1177/1535370217750088

[19] Soliman S, Mohan C. Lupus nephritis biomarkers. Clinical Immunology. 2017;**185**:10-20. DOI: 10.1016/j.clim.2016.08.001

[20] Stanley S, Vanarsa K, Soliman S, Habazi D, Pedroza C, Gidley G, et al. Comprehensive aptamer-based screening identifies a spectrum of urinary biomarkers of lupus nephritis across ethnicities. Nature Communications. 2020;**11**(1):2197. DOI: 10.1038/s41467-020-15986-3

[21] Palazzo L, Lindblom J, Mohan C, Parodis I. Current insights on biomarkers in lupus nephritis: A systematic review of the literature. Journal of Clinical Medicine. 2022;**11**(19):5759. DOI: 10.3390/jcm11195759

[22] For the IMID Consortium, Alonso A, Julià A, Vinaixa M, Domènech E, Fernández-Nebro A, et al. Urine metabolome profiling of immune-mediated inflammatory diseases. BMC Medicine. 2016;**14**(1):133. DOI: 10.1186/s12916-016-0681-8

[23] Romick-Rosendale LE, Brunner HI, Bennett MR, Mina R, Nelson S, Petri M, et al. Identification of urinary metabolites that distinguish membranous lupus nephritis from proliferative lupus nephritis and focal segmental glomerulosclerosis. Arthritis Research & Therapy. 2011;**13**(6):R199. DOI: 10.1186/ar3530

[24] Gergely P Jr, Grossman C, Niland B, Puskas F, Neupane H, Allam F, et al. Mitochondrial hyperpolarization and ATP depletion in patients with systemic lupus erythematosus. Arthritis and Rheumatism. 2002;**46**(1):175-190. DOI: 10.1002/1529-0131(200201)46:1<175::AID-ART10015>3.0.CO;2-H

[25] Perl A, Hanczko R, Doherty E. Assessment of mitochondrial dysfunction in lymphocytes of patients with systemic lupus erythematosus. Methods in Molecular Biology Clifton NJ. 2012;**900**:61-89. DOI: 10.1007/978-1-60761-720-4_4

[26] Yan B, Huang J, Zhang C, Hu X, Gao M, Shi A, et al. Serum metabolomic profiling in patients with systemic lupus erythematosus by GC/MS. Modern Rheumatology. 2016;**26**:1-25. DOI: 10.3109/14397595.2016.1158895

[27] Zhang Q, Li X, Yin X, Wang H, Fu C, Wang H, et al. Metabolomic profiling reveals serum L-pyroglutamic acid

as a potential diagnostic biomarker for systemic lupus erythematosus. Rheumatology Oxford England. 2021;**60**(2):598-606. DOI: 10.1093/rheumatology/keaa126

[28] Zhang Q, Yin X, Wang H, Wu X, Li X, Li Y, et al. Fecal metabolomics and potential biomarkers for systemic lupus erythematosus. Frontiers in Immunology. 2019;**3**(10):976. DOI: 10.3389/fimmu.2019.00976. DOI: 10.3389/fimmu.2019.00976

[29] Wu T, Xie C, Han J, Ye Y, Weiel J, Li Q, et al. Metabolic disturbances associated with systemic lupus erythematosus. PLoS One. 2012;**7**(6):e37210. DOI: 10.1371/journal.pone.0037210

[30] Cagnoli P, Harris RE, Frechtling D, Berkis G, Gracley RH, Graft CC, et al. Reduced insular glutamine and N-acetylaspartate in systemic lupus erythematosus: A single-voxel (1)H-MR spectroscopy study. Academic Radiology. 2013;**20**(10):1286-1296. DOI: 10.1016/j.acra.2013.07.011

[31] Brown J, Abboud G, Ma L, Choi SC, Kanda N, Zeumer-Spataro L, et al. Microbiota-mediated skewing of tryptophan catabolism modulates CD4+ T cells in lupus-prone mice. iScience. 2022;**25**(5):104241. DOI: 10.1016/j.isci.2022.104241

[32] Perl A, Hanczko R, Lai ZW, Oaks Z, Kelly R, Borsuk R, et al. Comprehensive metabolome analyses reveal N-acetylcysteine-responsive accumulation of kynurenine in systemic lupus erythematosus: Implications for activation of the mechanistic target of rapamycin. Metabolomics. 2015;**11**(5):1157-1174. DOI: 10.1007/s11306-015-0772-0

[33] He J, Chan T, Hong X, Zheng F, Zhu C, Yin L, et al. Microbiome and metabolome analyses reveal the disruption of lipid metabolism in systemic lupus erythematosus. Frontiers in Immunology. 2020;**31**(11):1703. DOI: 10.3389/fimmu.2020.01703

[34] Fernandez D, Bonilla E, Phillips P, Perl A. Signaling abnormalities in systemic lupus erythematosus as potential drug targets. Endocrine, Metabolic & Immune Disorders Drug Targets. 2006;**6**(4):305-311. DOI: 10.2174/187153006779025748

[35] Perl A. Systems biology of lupus: Mapping the impact of genomic and environmental factors on gene expression signatures, cellular signaling, metabolic pathways, hormonal and cytokine imbalance, and selecting targets for treatment. Autoimmunity. 2010;**43**(1):32-47. DOI: 10.3109/08916930903374774

[36] Shah D, Sah S, Nath SK. Interaction between glutathione and apoptosis in systemic lupus erythematosus. Autoimmunity Reviews. 2013;**12**(7):741-751. DOI: 10.1016/j.autrev.2012.12.007

[37] Yang Z, Matteson EL, Goronzy JJ, Weyand CM. T-cell metabolism in autoimmune disease. Arthritis Research & Therapy. 2015;**17**(1):29. DOI: 10.1186/s13075-015-0542-4

[38] Kono M, Yoshida N, Tsokos GC. Amino acid metabolism in lupus. Frontiers in Immunology. 2021;**12**:623844. DOI: 10.3389/fimmu.2021.623844

[39] Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. The Journal of Nutrition. 2004;**134**(3):489-492. DOI: 10.1093/jn/134.3.489

[40] Johnson MO, Wolf MM, Madden MZ, Andrejeva G, Sugiura A, Contreras DC, et al. Distinct regulation

of Th17 and Th1 cell differentiation by glutaminase-dependent metabolism. Cell. 2018;**175**(7):1780-1795.e19. DOI: 10.1016/j.cell.2018.10.001

[41] Fernandez DR, Telarico T, Bonilla E, Li Q, Banerjee S, Middleton FA, et al. Activation of mammalian target of rapamycin controls the loss of TCRzeta in lupus T cells through HRES-1/Rab4-regulated lysosomal degradation. Journal of Immunology. 2009;**182**(4):2063-2073. DOI: 10.4049/jimmunol.0803600

[42] Lai ZW, Hanczko R, Bonilla E, Caza TN, Clair B, Bartos A, et al. N-acetylcysteine reduces disease activity by blocking mammalian target of rapamycin in T cells from systemic lupus erythematosus patients: A randomized, double-blind, placebo-controlled trial. Arthritis and Rheumatism. 2012;**64**(9):2937-2946. DOI: 10.1002/art.34502

[43] Piranavan P, Bhamra M, Perl A. Metabolic targets for treatment of autoimmune diseases. Immunometabolism. 2020;**2**(2):e200012. DOI: 10.20900/immunometab20200012

[44] Perl A. Review: Metabolic control of immune system activation in rheumatic diseases. Arthritis & Rheumatology (Hoboken NJ). Dec 2017;**69**(12):2259-2270. DOI: 10.1002/art.40223

[45] Song W, Yuan J, Zhang Z, Li L, Hu L. Altered glutamate cysteine ligase activity in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. Experimental and Therapeutic Medicine. 2014;**8**(1):195-200. DOI: 10.3892/etm.2014.1689

[46] Duarte-Delgado NP, Cala MP, Barreto A, Rodríguez CLS. Metabolites and metabolic pathways associated with rheumatoid arthritis and systemic lupus erythematosus. Journal of Translational Autoimmunity. 2022;**5**:100150. DOI: 10.1016/j.jtauto.2022.100150

[47] Van der Werf P, Orlowski M, Meister A. Enzymatic conversion of 5-oxo-L-proline (L-pyrrolidone carboxylate) to L-glutamate coupled with cleavage of adenosine triphosphate to adenosine diphosphate, a reaction in the -glutamyl cycle. Proceedings of the National Academy of Sciences of the United States of America. 1971;**68**(12):2982-2985. DOI: 10.1073/pnas.68.12.2982

[48] Sitkovsky M, Lukashev D, Deaglio S, Dwyer K, Robson SC, Ohta A. Adenosine A2A receptor antagonists: Blockade of adenosinergic effects and T regulatory cells. British Journal of Pharmacology. 2008;**153**(Suppl 1):S457-S464. DOI: 10.1038/bjp.2008.23

[49] Mandapathil M, Hilldorfer B, Szczepanski MJ, Czystowska M, Szajnik M, Ren J, et al. Generation and accumulation of immunosuppressive adenosine by human CD4+ CD25highFOXP3+ regulatory T cells. The Journal of Biological Chemistry. 2010;**285**(10):7176-7186. DOI: 10.1074/jbc.M109.047423

[50] Gao ZW, Zhao GH, Zhang Z, Huang J, Li ZY, Zhang HZ, et al. Serum adenosine deaminase activity is increased in systemic lupus erythematosus patients and correlated with disease activity. Immunologic Research. 2018;**66**(2):299-304. DOI: 10.1007/s12026-018-8984-9

[51] Alunno A, Bartoloni E, Bistoni O, Nocentini G, Ronchetti S, Caterbi S, et al. Balance between regulatory T and Th17 cells in systemic lupus erythematosus: The old and the new. Clinical & Developmental Immunology. 2012;**2012**:823085. DOI: 10.1155/2012/823085

[52] Li Y, Wang HF, Li X, Li HX, Zhang Q, Zhou HW, et al. Disordered intestinal microbes are associated with the activity of systemic lupus erythematosus. Clinical Science London England 1979. 2019;**133**(7):821-838. DOI: 10.1042/CS20180841

[53] Colgan SP, Fennimore B, Ehrentraut SF. Adenosine and gastrointestinal inflammation. Journal of Molecular Medicine Berl Ger. 2013;**91**(2):157-164. DOI: 10.1007/s00109-012-0990-0

[54] Haskó G, Cronstein B. Regulation of inflammation by adenosine. Frontiers in Immunology. 2013;**8**(4):85. DOI: 10.3389/fimmu.2013.00085

[55] Kono M, Yoshida N, Maeda K, Suárez-Fueyo A, Kyttaris VC, Tsokos GC. Glutaminase 1 inhibition reduces glycolysis and ameliorates lupus-like disease in MRL/lpr mice and experimental autoimmune encephalomyelitis. Arthritis & Rheumatology Hoboken NJ. 2019;**71**(11):1869-1878. DOI: 10.1002/art.41019

[56] DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, et al. Beyond aerobic glycolysis: Transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(49):19345-19350. DOI: 10.1073/pnas.0709747104)

[57] Wang XY, Li N, Gu J, Li WQ, Li JS. The effects of the formula of amino acids enriched BCAA on nutritional support in traumatic patients. World Journal of Gastroenterology. 2003;**9**(3):599-602. DOI: 10.3748/wjg.v9.i3.599

[58] Shi H, Chapman NM, Wen J, Guy C, Long L, Dhungana Y, et al. Amino acids license kinase mTORC1 activity and Treg cell function via small G proteins rag and Rheb. Immunity. 2019;**51**(6):1012-1027.e7. DOI: 10.1016/j.immuni.2019.10.001

[59] Kato H, Perl A. Mechanistic target of rapamycin complex 1 expands Th17 and IL-4+ CD4-CD8- double-negative T cells and contracts regulatory T cells in systemic lupus erythematosus. Journal of Immunology Baltimore Md. 2014;**192**(9):4134-4144. DOI: 10.4049/jimmunol.1301859

[60] Zheng Y, Delgoffe GM, Meyer CF, Chan W, Powell JD. Anergic T cells are metabolically anergic. Journal of Immunology Baltimore Md. 2009;**183**(10):6095-6101. DOI: 10.4049/jimmunol.0803510

[61] Ananieva EA, Patel CH, Drake CH, Powell JD, Hutson SM. Cytosolic branched chain aminotransferase (BCATc) regulates mTORC1 signaling and glycolytic metabolism in CD4+ T cells. The Journal of Biological Chemistry. 2014;**289**(27):18793-18804. DOI: 10.1074/jbc.M114.554113

[62] Papathanassiu AE, Ko JH, Imprialou M, Bagnati M, Srivastava PK, Vu HA, et al. BCAT1 controls metabolic reprogramming in activated human macrophages and is associated with inflammatory diseases. Nature Communications. 2017;**12**(8):16040. DOI: 10.1038/ncomms16040

[63] Zhang T, Mohan C. Caution in studying and interpreting the lupus metabolome. Arthritis Research & Therapy. 2020;**22**:172. DOI: 10.1186/s13075-020-02264-2

[64] Ardawi MS. Glutamine and glucose metabolism in human peripheral lymphocytes. Metabolism. 1988;**37**(1):99-103. DOI: 10.1016/0026-0495(88)90036-4

[65] Nakaya M, Xiao Y, Zhou X, Chang JH, Chang M, Cheng X, et al. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. Immunity. 2014;**40**(5):692-705. DOI: 10.1016/j.immuni.2014.04.007

[66] Yoshida N, Comte D, Mizui M, Otomo K, Rosetti F, Mayadas TN, et al. ICER is requisite for Th17 differentiation. Nature Communications. 2016;**29**(7):12993. DOI: 10.1038/ncomms12993

[67] Kono M, Yoshida N, Maeda K, Tsokos GC. Transcriptional factor ICER promotes glutaminolysis and the generation of Th17 cells. Proceedings of the National Academy of Sciences of the United States of America. 2018;**115**(10):2478-2483. DOI: 10.1073/pnas.1714717115

[68] Leung LH. Systemic lupus erythematosus: A combined deficiency disease. Medical Hypotheses. 2004;**62**(6):922-924. DOI: 10.1016/j.mehy.2004.01.017

[69] Oaks Z, Winans T, Huang N, Banki K, Perl A. Activation of the mechanistic target of rapamycin in SLE: Explosion of evidence in the last five years. Current Rheumatology Reports. 2016;**18**(12):73. DOI: 10.1007/s11926-016-0622-8

[70] Zhang L, Qing P, Yang H, Wu Y, Liu Y, Luo Y. Gut microbiome and metabolites in systemic lupus erythematosus: Link, mechanisms and intervention. Frontiers in Immunology. 2021;**15**(12):686501. DOI: 10.3389/fimmu.2021.686501), 10.3389/fimmu.2021.686501)

[71] Hevia A, Milani C, López P, Cuervo A, Arboleya S, Duranti S, et al. Intestinal dysbiosis associated with systemic lupus erythematosus. MBio. 2014;**5**(5):e01548-e01514. DOI: 10.1128/mBio.01548-14

[72] He Z, Shao T, Li H, Xie Z, Wen C. Alterations of the gut microbiome in Chinese patients with systemic lupus erythematosus. Gut Pathogens. 2016;**8**:64. DOI: 10.1186/s13099-016-0146-9

[73] Azzouz D, Omarbekova A, Heguy A, Schwudke D, Gisch N, Rovin BH, et al. Lupus nephritis is linked to disease-activity associated expansions and immunity to a gut commensal. Annals of the Rheumatic Diseases. 2019;**78**(7):947-956. DOI: 10.1136/annrheumdis-2018-214856

[74] Chi M, Ma K, Wang J, Ding Z, Li Y, Zhu S, et al. The immunomodulatory effect of the gut microbiota in kidney disease. Journal of Immunology Research. 2021;**15**(2021):5516035. DOI: 10.1155/2021/5516035

[75] Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, et al. Gut microbiota functions: Metabolism of nutrients and other food components. European Journal of Nutrition. 2018;**57**(1):1-24. DOI: 10.1007/s00394-017-1445-8

[76] Mok CC. Metabolic syndrome and systemic lupus erythematosus: The connection. Expert Review of Clinical Immunology. 2019;**15**(7):765-775. DOI: 10.1080/1744666X.2019.1620601

[77] Parker B, Urowitz MB, Gladman DD, Lunt M, Bae SC, Sanchez-Guerrero J, et al. Clinical associations of the metabolic syndrome in systemic lupus erythematosus: Data from an international inception cohort. Annals of the Rheumatic Diseases. 2013;**72**(8):1308-1314. DOI: 10.1136/annrheumdis-2012-202106

[78] Demir S, Artim-Esen B, Şahinkaya Y, Pehlivan Ö, Alpay-Kanıtez N, Omma A, et al. Metabolic syndrome is not only a risk factor for cardiovascular diseases in systemic lupus erythematosus but is also associated with cumulative organ damage: A cross-sectional analysis of 311 patients. Lupus. 2016;**25**(2):177-184. DOI: 10.1177/0961203315603140

[79] Sarkissian T, Beyene J, Feldman B, McCrindle B, Silverman ED. Longitudinal examination of lipid profiles in pediatric systemic lupus erythematosus. Arthritis and Rheumatism. 2007;**56**(2):631-638. DOI: 10.1002/art.22332

[80] Sun W, Li P, Cai J, Ma J, Zhang X, Song Y, et al. Lipid metabolism: Immune regulation and therapeutic prospectives in systemic lupus erythematosus. Frontiers in Immunology. 2022;**18**(13): 860586. DOI: 10.3389/fimmu.2022. 860586)

[81] Li Y, Liang L, Deng X, Zhong L. Lipidomic and metabolomic profiling reveals novel candidate biomarkers in active systemic lupus erythematosus. International Journal of Clinical and Experimental Pathology. 2019;**12**(3):857-866

[82] Frostegård J, Svenungsson E, Wu R, Gunnarsson I, Lundberg IE, Klareskog L, et al. Lipid peroxidation is enhanced in patients with systemic lupus erythematosus and is associated with arterial and renal disease manifestations. Arthritis and Rheumatism. 2005;**52**(1): 192-200. DOI: 10.1002/art.20780

[83] Hu C, Du Y, Xu X, Li H, Duan Q, Xie Z, et al. Lipidomics revealed aberrant metabolism of lipids including FAHFAs in renal tissue in the progression of lupus nephritis in a murine model. Metabolites. 2021;**11**(3):142. DOI: 10.3390/ metabo11030142

[84] Frostegård J. SLE, atherosclerosis and cardiovascular disease. Journal of Internal Medicine. 2005;**257**(6):485-495. DOI: 10.1111/j.1365-2796.2005.01502.x

[85] Robinson GA, MGL W, Wincup C. The role of immunometabolism in the pathogenesis of systemic lupus erythematosus. Frontiers in Immunology. 2022;**26**(12):806560. DOI: 10.3389/ fimmu.2021.806560

[86] Kiss M, Czimmerer Z, Nagy L. The role of lipid-activated nuclear receptors in shaping macrophage and dendritic cell function: From physiology to pathology. The Journal of Allergy and Clinical Immunology. 2013;**132**(2):264-286. DOI: 10.1016/j.jaci.2013.05.044

[87] Ryu H, Lim H, Choi G, Park YJ, Cho M, Na H, et al. Atherogenic dyslipidemia promotes autoimmune follicular helper T cell responses via IL-27. Nature Immunology. 2018;**19**(6):583-593. DOI: 10.1038/ s41590-018-0102-6

[88] Waddington KE, Papadaki A, Coelewij L, Adriani M, Nytrova P, Kubala Havrdova E, et al. Using serum metabolomics to predict development of anti-drug antibodies in multiple sclerosis patients treated with IFNβ. Frontiers in Immunology. 2020;**17**(11):1527. DOI: 10.3389/fimmu.2020.01527

[89] Lian F, Wang Y, Chen J, Xu H, Yang X, Liang L, et al. Activation of farnesoid X receptor attenuates liver injury in systemic lupus erythematosus. Rheumatology International. 2012;**32**(6):1705-1710. DOI: 10.1007/ s00296-011-1874-2

[90] Hang S, Paik D, Yao L, Kim E, Trinath J, Lu J, et al. Bile acid metabolites control TH17 and Treg cell differentiation. Nature. 2019;**576**(7785):143-148. DOI: 10.1038/ s41586-019-1785-z

[91] Kalantari S, Chashmniam S, Nafar M, Zakeri Z, Parvin M. Metabolomics approach reveals urine biomarkers and pathways associated with the pathogenesis of lupus nephritis. Iranian Journal of Basic Medical Sciences. 2019;**22**(11):1288-1295. DOI: 10.22038/ijbms.2019.38713.9178)

[92] Souliotis VL, Vougas K, Gorgoulis VG, Sfikakis PP. Defective DNA repair and chromatin organization in patients with quiescent systemic lupus erythematosus. Arthritis Research & Therapy. 2016;**18**(1):182. DOI: 10.1186/s13075-016-1081-3

[93] Nowling TK, Mather AR, Thiyagarajan T, Hernández-Corbacho MJ, Powers TW, Jones EE, et al. Renal glycosphingolipid metabolism is dysfunctional in lupus nephritis. Journal of the American Society of Nephrology. 2015;**26**(6):1402-1413. DOI: 10.1681/ASN.2014050508

[94] Li J, Xie XW, Zhou H, Wang B, Zhang MJ, Tang FY. Metabolic profiling reveals new serum biomarkers of lupus nephritis. Lupus. 2017;**26**(11):1166-1173. DOI: 10.1177/0961203317694256

[95] Ganguly S, Kumar U, Gupta N, Guleria A, Majumdar S, Phatak S, et al. Nuclear magnetic resonance–based targeted profiling of urinary acetate and citrate following cyclophosphamide therapy in patients with lupus nephritis. Lupus. 2020;**29**(7):782-786. DOI: 10.1177/0961203320918011

[96] Sui W, Hou X, Che W, Yang M, Dai Y. The applied basic research of systemic lupus erythematosus based on the biological omics. Genes and Immunity. 2013;**14**(3):133-146. DOI: 10.1038/gene.2013.3

[97] Guleria A, Pratap A, Dubey D, Rawat A, Chaurasia S, Sukesh E, et al. NMR based serum metabolomics reveals a distinctive signature in patients with lupus nephritis. Scientific Reports. 2016;**6**(1):35309. DOI: 10.1038/srep35309

[98] Ouyang X, Dai Y, Wen J, Wang L. ^{1}H NMR-based metabolomic study of metabolic profiling for systemic lupus erythematosus. Lupus. 2011;**20**(13):1411-1420. DOI: 10.1177/0961203311418707

[99] Watanabe M, Suliman ME, Qureshi AR, Garcia-Lopez E, Bárány P, Heimbürger O, et al. Consequences of low plasma histidine in chronic kidney disease patients: Associations with inflammation, oxidative stress, and mortality. The American Journal of Clinical Nutrition. 2008;**87**(6):1860-1866. DOI: 10.1093/ajcn/87.6.1860

Chapter 7

Perspective Chapter: Making the Shift to Personalized Preventive Medicine with Human Digital Twins

Nabil Abu el Ata

Abstract

Human digital twins (HDTs) have the potential to support a paradigm shift from one-size-fits-all sick care to highly personalized preventive healthcare. By providing important context for complex disease processes and enhancing our understanding of the dynamic interactions that lead to non-communicable diseases (NCDs), HDTs are poised to offer researchers, care providers, and public health agencies the toolset they need to predictively diagnose and treat NCDs with highly customized interventions. The precision health knowledge gained from HDTs can help patients understand their NCD risks, public health authorities support care pathways that effectively prevent or delay the onset of chronic diseases, and care providers prescribe interventions based on an individual's unique biological, behavioral, and environmental characteristics. This chapter presents key human digital twin concepts and model performance evaluation criteria. Digital twin applications in preventive medicine research, clinical care, and public health are presented while acknowledging the associated challenges, including model robustness and ethical concerns surrounding the use of digital twins to model humans.

Keywords: human digital twin, virtual twin, preventive medicine, personalized medicine, non-communicable diseases, multimorbidity, precision public health

1. Introduction

Our current approach to healthcare is unsustainable. Favoring one-size-fits-all reactive sick care above preventive healthcare creates societal and economic burdens. Globally, NCDs, including heart disease, stroke, cancer, diabetes, and chronic lung disease, are the leading causes of death and disability. According to the World Health Organization (WHO), NCDs are collectively responsible for 74% of all deaths worldwide, resulting in 15 million people dying between the ages of 30–69 years each year [1].

Even though an estimated 80% of NCDs are preventable [2], the global NCD burden is expected to increase by 17% over the next 10 years [3]. The coronavirus (COVID-19) pandemic has amplified the global burden of NCDs. Based on available

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data from multiple countries, it is evident that people with underlying NCDs such as hypertension, diabetes, cardiovascular disease, chronic respiratory disease, and cancer have a higher risk of severe COVID-19 disease and are more likely to die from it [4].

Individuals, families, businesses, governments, and economies, directly and indirectly, bear the brunt of NCDs through high treatment costs and productivity losses caused by premature mortality, early labor force exits, absenteeism, and lowered work capacity [5]. The cost of NCDs to healthcare systems currently accounts for almost half of the general hospital expenditures in most developed countries [6, 7]. Over the last two decades, healthcare spending on diseases like cancer [8] and diabetes [9] has increased faster than disease incidences.

Despite global commitments to control NCDs, preventive care is underfunded, and nations lack the policies and interventions to effectively prevent and manage chronic diseases. In the U.S., Europe, and other wealthy countries, preventive care averaged 2.4% of total public and private healthcare expenditure [10]. As with any medical expenditure, the value of prevention is judged based on the benefit it provides, measured by improved quality of life, productivity, or both. To improve preventive medicine's cost-to-benefit ratio, control programs should focus on pre-onset of disease and pre-pathogenesis activities. Spending on secondary and tertiary prevention occurs too late—after destructive physical and mental effects have impacted the patient's productivity and quality of life. Once an NCD is diagnosed, treatments are generally less effective, more complex, and more expensive. As example, treatment for cancer patients diagnosed early is 2–4 times less expensive than treating people diagnosed with cancer at more advanced stages [11].

Identifying at-risk patients before disease onset using current state-of-the-art protocols is difficult. Uncertainty persists when translating research data into evidence-based precision medicine because the average outcome of carefully chosen study participants may not represent a specific patient—especially in complex and ambiguous cases. Even with current NCD behavior modification consulting and screening programs, doctors commonly cannot diagnose or treat NCDs until symptoms appear. Sometimes, disease diagnosis is further delayed if symptoms are common to less severe conditions or the case is complex due to multimorbidity or other compounding factors.

Precision medicine strives to reduce inaction or waste by applying more granular population stratification to disease control programs. But without causal knowledge, filling the gaps between epidemiology research and clinical practice is challenging. The results of epidemiological studies designed to determine patient risks for common chronic ailments such as cardiovascular, cancer, diabetes, and neurodegenerative diseases tend to produce marginal, contradictory, irreproducible, or hard-to-interpret results [12].

Evidence suggests that more screening does not always translate into fewer deaths [13]. For example, over 1300 women aged 50–59 need a mammogram to save one life [13]. Some screenings lead to relatively small reductions in mortality. One study found that lung cancer screenings yield a 0.4% reduction in patient mortality rates [13]. Further screenings can lead to unnecessary follow-up screening tests, anxiety caused by false-positive results, and strains on resources due to the overdiagnosis of diseases [13]. Studies show that 19% of screen-detected breast cancers [14] and 20–50% of screen-detected prostate cancers are overdiagnosed [15].

Population-based statistics can also lead to inaction due to personal beliefs, attitudes, or fear of a diagnosis. For example, research findings suggest only 10–20% of smokers will develop lung cancer. Patients may choose to continue smoking and avoid lung cancer screening programs—believing they will be in the 80–90% of the population who smoke but do not develop cancer.

With innovations, such as genomics, slowly getting integrated into care, the complexity of NCD knowledge is only increasing. At the same time, the acumen of doctors is decreasing because their longitudinal experience with patients is decreasing. Which means doctors do not see their mistakes play out.

Over the last decade, many within the medical community have recognized the need to complement traditional research with system-based approaches to support the goals of personalized preventive medicine [16–22]. Many theoretical frameworks have been proposed, and yet, most practical progress is still achieved through reductionist processes that heavily rely on statistical data to build models that explain and discover functions at a single system level (e.g., gene, protein, cellular, tissue, or organ level) [16]. Using the resulting circumstantial data as inputs for machine learning (ML) and large language models (LLM), only introduces more, not less, uncertainty in preventive medicine due to standard errors, data integrity issues, model bias, and unexplainable results [23].

Human digital twins (HDTs) support a paradigm shift to personalized right-time preventive care by adding the missing context of complex disease processes and promoting further understanding of how dynamic interactions between thousands of determinants impact a patient's susceptibility to disease. Such efforts aim to build an iterative process that combines epidemiological research with digital twin technologies to deepen the understanding of disease progression and cognitively discover promising new areas for scientific research.

The precision health knowledge gained from HDTs can be used to extrapolate predictions about disease behaviors not covered by conventional data-based deductions. Scientists can use HDTs to accelerate the translation of research into effective primordial and primary precision preventive protocols. Representative virtual models of patients can help doctors make clinical care decisions based on a patient's unique circumstances and predicted health risks. Explainable, personalized health metrics can empower people to take control of their health. For public health authorities, HDTs can prove the benefits of preventive healthcare programs, inform policy choices, and support highly deterministic clinical pathways characterized by high reproducibility of care.

2. Causality is key to personalizing prevention

NCDs are dynamic processes that evolve through environmental factors, genetic predisposition, disease agents, and lifestyle choices. Interrelations among the components of health often include dynamic challenges, such as feedback loops and changes over time that cannot be understood using linear or reductionist paradigms. Often, two or more patients with the same disease are very different regarding causes, evolution, and temporal conditions. These dynamic differences are illustrated in COVID-19 patients and other autoimmune diseases. Further, epidemiological research has demonstrated the strong connections between seemingly disparate diseases, as evidenced by the number of patients who share diagnoses with two or more "unrelated" disease processes [24].

Complexity science asserts that to develop a more comprehensive and complete understanding of the whole, it is necessary to reinforce the importance of understanding how each part interacts with all the other parts and emerges into something new. A key concept of systems theory is that the whole is greater than the sum of its parts—meaning that when holistically examining how smaller systems

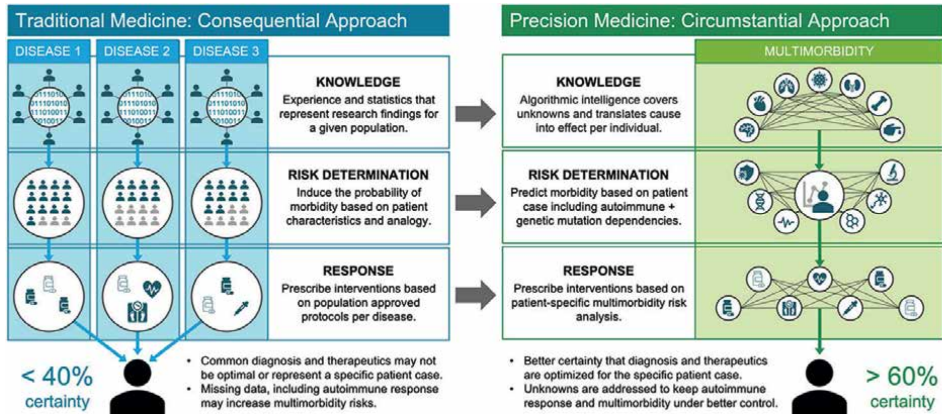

Figure 1.
Difference between consequential vs. circumstantial approach to NCD prevention.

come together to affect the entire complex system, specific characteristics of the whole cannot be easily explained or rationalized when looking singularly at any one of its parts.

Therefore, the goal of precision preventive medicine cannot be fully obtained without a method to model a human's health dynamics, constraints, and conditions and then elucidate principles (such as purpose, measure, methods, and tools) in ways that can be applied to understand and predict NCD behaviors for other humans. This requires the formulation of predictive mathematical and computational models that cover the functional, graph connectivity, and regulatory networks and are as complex as necessary to account for specific details while being computationally tractable [16]. **Figure 1** summarizes the differences between the current consequential versus the proposed circumstantial approach to epidemiology.

To reproduce system-wide behavior and uncover the origins of known as well as unknown behaviors, three factors must be considered: (1) context, which covers the values that represent all components related to the process being studied; (2) time, which considers the changing characteristics of each component; and (3) space, which accounts for the topographic relationships between and among components [19]. This approach naturally leads to the development of mechanistic models that support the analysis of one patient at a time instead of averages across large patient populations. If a model combines a large group of people, many characteristics that make an individual unique must be ignored; otherwise, the solution becomes computationally intractable.

2.1 Hierarchical model

The mechanisms of human health are hierarchical in that lower-level dependencies influence and decompose higher-level behaviors. Therefore, to adequately draw quantitative predictions, the model needs to capture these hierarchical relationships horizontally within and vertically between multiple levels. Further, health is not a closed system. At any point, a patient's health is influenced by external factors such as the environment, individual behaviors, access to healthcare, and socioeconomics (see **Figure 2**).

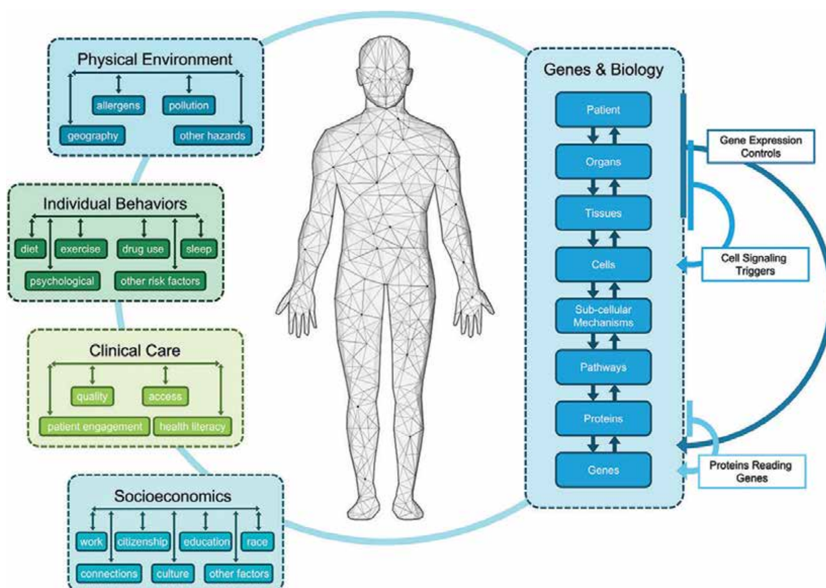

Figure 2.
Simplified diagram of human health determinants and biological causal relationships.

2.2 Nonlinear dynamics

A nonlinear system is defined as a system in which the change of the output is not proportional to the change of the input. Natural and living systems' flexibility and ability to adapt to and cope with different conditions originate from this nonlinearity [25]. As such, nonlinearity plays a fundamental role in the performance, functionality, and evolution of health. Consequently, understanding nonlinearity is vital to accurately predicting the pre-onset of diseases and pre-pathogenesis conditions for an individual.

Since the 1800s, Claude Bernard and then Walter B. Cannon popularized homeostasis as a guiding principle of medicine—emphasizing the body's remarkable ability to maintain stability and constancy in the face of stress [26]. From this point of view, illness is defined as a failed homeostatic mechanism, and treatment aims to correct health failures by reestablishing parameters within a normal range. As such, the homeostasis paradigm has placed a significant emphasis on static stability (i.e., normal ranges) and not on dynamic stable states, such as oscillatory [27] or chaotic behavior [28–32].

Further, homeostasis implies that human health operates as a closed-loop system when in fact, human health is better defined as an evolutionary system, which perpetually changes over time in response to interactions with the environment and surrounding circumstances. The evolution of health (or disease) takes multiple forms, and the predictability of system behaviors is difficult to explain by linking a cause to an observed effect when using historical statistical data, which was only valid when it was captured. In many medical models, data extraction, e.g., obtaining serum glucose level or blood pressure, creates a loss of time, space, or context information. This leads to the loss of rich information that, if captured, would help

improve understanding of the systemic and dynamic behavior of the human body [19]. Disease progression and mutations provide examples of chaotic behaviors that are difficult to prevent and/or treat without the concept of nonlinearity in medicine.

Failure to include dynamic states in models can often lead to treatments that are either ineffective or detrimental [19]. Many scientists and clinicians have largely ignored that less intuitive treatments may yield more effective outcomes or that the correction itself may invoke harmful system-wide effects [33–35]. To address this shortcoming, it must be recognized that the behavior of a nonlinear system can be sensitive to the system's parameters and initial conditions. More specifically, by changing a nonlinear system's parameters, the system's behavior can change qualitatively, as well as quantitatively—and often dramatically, especially in the case of chronic diseases.

Studies have shown that chaos is a widespread phenomenon throughout the biological hierarchy, ranging from simple enzyme reactions to entire ecosystems [36]. Mutation demonstrates the nonlinearity of health. For example, variants of SARS-CoV-2 are caused by a mutation at the protein level [37], and mutations in the DNA sequence contribute to cancer progression [38]. The dynamics associated with a mutation can cause a patient's health to become unstable. Therefore, the goal of preventive medicine should be to sufficiently understand chaotic, evolutionary, and transformative processes so that it becomes possible to predict their occurrence and control the associated risks.

2.3 Identifying the right mathematical solution

Auffray et al. assert that most mathematical theories are unsuitable to deal with the nonlinearity of health because they do not fully address the space and time scales characteristic of biological systems and cannot make experimentally testable predictions relevant to fundamental biological questions [16]. However, even though some aspects of a nonlinear system's dynamic behaviors can appear counterintuitive, unpredictable, or chaotic, the behavior is not random. Therefore, the right mathematical expression, paired with adequate solvers and supporting digitalization technologies, should support the prediction of system behaviors with a high degree of accuracy [30, 31].

A mathematically robust solution is necessary to build a representative model because the variations of parameter constituents of NCDs are considerable. To support a move towards systems medicine, many proposed models use network principles [39] that describe relations and behaviors of elements that reside in a common spatial level (or layer) of the system being studied [40]. Further, the reliance on massive amounts of data, Bayesian logic, machine learning, and numerical solutions introduce a priori knowledge, uncertainty, spatiotemporal dependence, and/or computational challenges [23].

Mechanistic mathematical models provide a viable way to overcome the limitations of statistical and numerical solutions. An added advantage of a mechanistic approach is that the resulting model can be used repeatedly across multiple medical applications, whereas statistical and numerical models must be continually reconfigured to cover any change in time or space. Typically, mechanistic models within medical sciences have been perceived as too simplistic to cover the full scope of complex systems and sub-systems that represent health [41]. However, other sciences, such as elementary particle physics and gravitational mechanics [42–44], have shown it is possible to use mechanistic mathematical models to cover a full hierarchy of

perturbed graphs as required to discover the underlying dynamics of systems—even from sparse or noisy experimental data [45, 46].

A systems-based framework and perturbation mathematical foundations can add the missing context of complex disease processes and promote further understanding of how dynamic interactions between thousands of determinants, such as DNA [47], pathogens [18], autoimmune responses [48], metabolism [49], and aging [50] influence an individual's health.

2.4 Why use a mechanistic model?

A mechanistic mathematical model can be defined as the mathematical description of the elements forming a system, their mutual interactions, and their interaction with the environment [51]. Mathematical modeling, emulation, and optimization are already used in systems medicine, and mechanistic modeling is currently the main focus [51–53].

The purpose of a mechanistic model is to mimic real-life events by making assumptions about the underlying mechanisms and refining the model until the desired accuracy is achieved. Typically, this involves constructing mathematical formulations of causal mechanisms and using analytical tools to determine whether the range of possible input-output behaviors predicted by the model is consistent with experimental observations.

Mechanistic modeling relies upon a two-stage process: (1) a subset of the available data is used to construct and calibrate the model, and (2) in a validation phase, further data is used to confirm and/or refine the model, thereby increasing its accuracy. The advantage of using a mechanistic model instead of a data-driven or machine learning/black box model is that the mechanistic model can address the complexity of human biology and the lack of detailed information about biological system elements by supporting what-if scenarios as well as the ability to confirm the representativeness of the model through targeted emulation exercises and randomized experiments.

When modeling a system as complex as human health, the modeler lacks empirical data to explain critical phenomena through which independent variables interact to produce complex and synergetic nonlinear effects. Statistical methods attempt—without success in most cases—to develop a fundamental understanding of the root causes that impact the most crucial factors within a system using probabilistic treatment of production data or experimental results.

Finding mathematical solutions that enable modelers to improve the certainty of predictions and cover the unknowns is critical to advancing the goals of many areas of scientific study, as well as artificial intelligence and machine learning. This is especially true for decisions that may have a profound impact on human life and well-being. It is, therefore, essential to have mathematical solutions that can expose and define the appropriate mitigating actions for scenarios that may not be represented by experience or historical data.

2.5 Combined use of graph and perturbation theory

Graphs are mathematical structures used to model pairwise relations between objects. Graph theory provides a mathematical nonlinear data structure capable of representing various kinds of physical structure—consisting of a group of vertices (or nodes) and a set of edges that connect the two vertices. In practical applications, vertices and edges of graphs often contain specific information, such as labels or weights.

Many real-life scenarios are better modeled by time-dependent graphs when sequences of time-dependent elements activate the edges [54]. For instance, in bioinformatics networks, graphs reflect the similarity and regulation of biomolecules, such as proteins, genes, and enzymes [55–57]. The connections in biological functions are not always active, but the status may change over time [58, 59].

The proposed perturbed graph solution's structure is like those applied in quantum and celestial mechanics [60]. As such, this method can be considered a generalized approach that provides the foundation to find an acceptable approximation for an exact, unperturbed solution. The solution is complex because the problem at hand is complex—both as a product of dynamics/nonlinearity and because the determining factors of health behaviors may be found anywhere in the hierarchal structure of the human being studied. Horizontally, dependencies may drive behaviors; vertically, behaviors may result from direct and indirect causes. Finding these causes is sometimes more important than finding the unperturbed solution itself.

Using perturbation theory and the associated mathematical equations as the basis for building a mechanistic model of patient health provides an analytical framework that allows practitioners to build an approximation method for separable structures and small divisors. The small divisors generally appear late in analytical expansions as inequalities that seem trivial but can produce significant contributions to the final solution. The small divisors often represent chaotic behaviors missing from traditional research but are nevertheless vital in understanding the causality of complex disease processes. Therefore, new insights about their influence on health can be gained by identifying these small divisors and measuring their effect on the perturbed solution.

Compared to alternative modeling methods, perturbation theory provides the framework necessary to capture and reduce the complexity and maintain computational control of the equations. It is, therefore, possible to represent the entire spatiotemporal evolution of disease processes efficiently and accurately, such as multimorbidity, without creating ill-posed problems or non-physically acceptable solutions.

3. Personalizing prevention with HDT

A digital twin, also called a virtual twin, is a software representation of a real-world asset, system, or process designed to detect, prevent, predict, and optimize the system being studied through mathematical or statistical analysis. Many industries are using digital twins to improve the performance of real-world systems through computational analysis of a virtual counterpart [48]. Human digital twins (HDTs) extend the concept of digital twins to cover the mathematical replication of human health process dynamics.

HDTs built from graph-based perturbation theory can encourage a better understanding of how thousands of dynamically complex determinants interact horizontally and vertically across multiple scales to influence an individual's predicted NCD risk or effectiveness of interventions. When used as an experimentation and decision-validation platform, HDTs may offer researchers, care providers, and public health agencies the toolset they need to predictively prevent NCDs through highly customized interventions.

3.1 HDT requirements

To support accurate prediction for an individual, HDTs must start with a representative model that accurately captures the dynamics of health based on the following criteria:

- Patient-specific: To factor in how genes, autoimmune responses, environment, and lifestyle choices may influence morbidity for an individual patient, HDT models should be computationally efficient and robust enough to analyze many-to-many causal relationships between millions of interdependent and time-sensitive biological and nonbiological health factors.

- *Accurate prediction of dynamic behaviors*: HDTs should allow users to calculate a future event precisely without the involvement of randomness. Deterministic models can limit uncertainty in clinical applications because they provide a mathematical representation in which every variable alters according to a mathematical formula. This makes it possible to trust predictions about behaviors not covered by conventional data.

- *Reproducible*: The model should always produce the same output from a given starting condition or initial state. Reproducibility makes it possible to repeatedly use the model across multiple medical applications, whereas probabilistic models must be continually reconfigured to cover any change in time or space.

- *Explainable*: Finding correlations is not the same as proving causation. ML based on statistical or correlation studies bypasses the need for causality and focuses exclusively on prediction. This leads to unexplainable results that are difficult to trust for critical health decisions. Mechanistic models focus on the causality of input-output relationships. This provides a way to explain the underlying dynamics of health—even when starting from sparse or noisy experimental data [45, 46].

3.2 Iterative HDT methodology

A symbiotic relationship between mechanistic models and machine learning can help accelerate the accrual of medical knowledge [23] and support the use of HDTs in research and clinical applications [61, 62]. Synthetic data, also called algorithmic cognition, derived from HDTs can be used by machine learning algorithms both as transient inputs and as validating frameworks, while machine learning can be harnessed to improve the scalability of mechanistic models (see **Figure 3**).

Algorithmic cognition or synthetic data combined with robotization will provide the flexibility necessary to account for the evolutionary process of morbidity and move precision preventive medicine from concept to value in research and clinical applications. For instance, ML can be used to query a patient database to predict which existing immunotherapy treatment will be most effective in preventing cancer for an individual patient based on past observations, but intrinsically, a learning algorithm cannot suggest new treatment protocols or accurately predict the outcome of new treatments [23]. When machine learning was used to predict the success rate for endoscopic third ventriculostomy as a treatment for hydrocephalus [63], the algorithm could predict the success rate of the actual procedure, but it was not able to

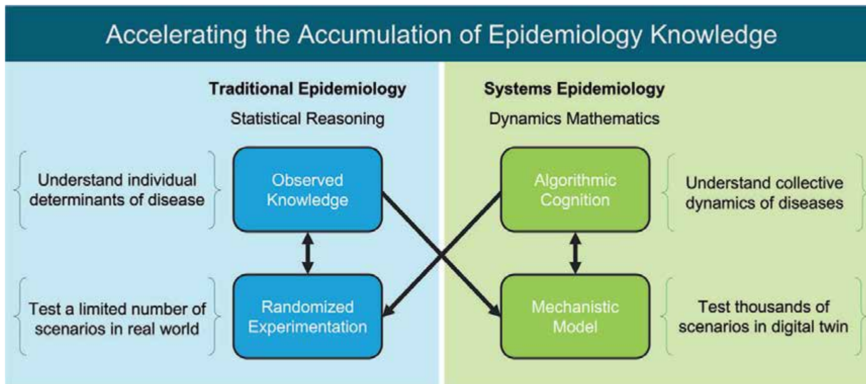

Figure 3.
Pair observed knowledge with algorithmic cognition to accelerate the accumulation of epidemiology knowledge.

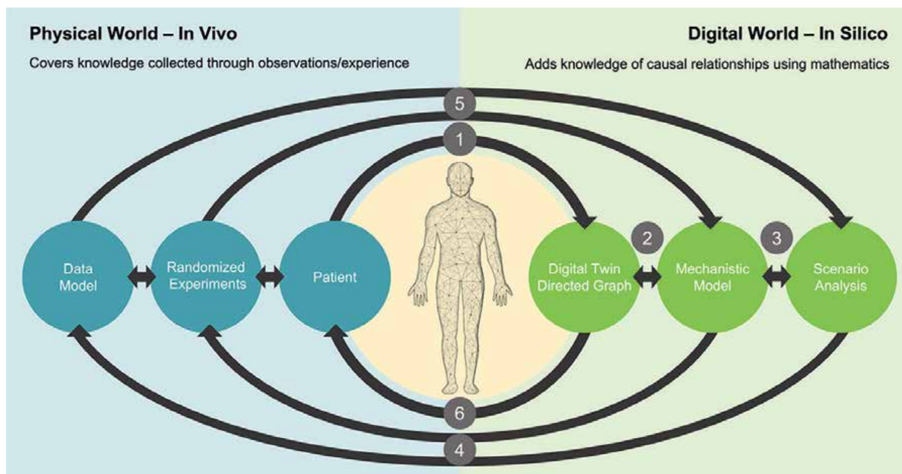

Figure 4.
Iterative analysis methodology for multidimensional medicine.

consider general physiological variables that may pose a risk for a particular patient and predict a more favorable outcome using a different procedure. In new or complex situations, the deductive capabilities of HDTs are needed to extrapolate behavior predictions that are not present in the original data [64] and consider all the specific dimensions for a particular patient versus the averages of a population of patients.

HDTs provide the framework necessary to holistically compare the patient's current state, future risks, and available treatment options that support the optimal outcome for a given patient. **Figure 4** outlines the 6 steps used to digitally transform the mechanisms of health into an HDT that covers the full hierarchy of graphs necessary to gain accuracy and certainty in behavior predictions that can be used for discovery and experimentation. The outcome of each cycle supports an improvement process whereby HDTs reveal the missing data, which can be vetted through randomized experimentation and data analysis. Then, once the algorithmic cognition is confirmed, ML can cover individual patient scenarios more exhaustively in broader research and clinical applications.

3.2.1 Step 1: deconstruct components of health

The first step to building an HDT is deconstructing human health using causal deconstruction theory [65] and network mapping methodology. This is necessary to understand the constituent components of health and their dependencies. The goal is to build a directed graph that maps the interdependencies, topology of structures, justification of choices, operational constraints, modes of operations, and data necessary to discover the hidden structures that form over time. **Figure 5** illustrates an example of a directed graph covering the complex interdependencies carried by an individual parameter as possible perturbations of multiple interacting networks for patient health.

3.2.2 Step 2: build a mechanistic model

From this knowledge, a generic HDT can be built using a perturbed graph solution (see **Figure 6**) [66]. The HDT should cover previously validated morbidity diagnoses, remediation, mitigation actions, and any influencing biological, public policy, healthcare, habits, or social and environmental factors that improve the HDT's ability to reliably predict NCD risks and identify possible interventions.

3.2.3 Step 3: perform scenario analysis

Once the HDT is constructed, sensitivity analysis and what-if analysis can be performed to determine how different values of an independent variable impact a dependent variable under a given set of assumptions. This analysis exposes the unknown influences of disease(s) or treatment processes. It delivers the synthetic knowledge necessary to rapidly identify a potential health risk with immediate analysis of root causes and proposed intervention.

By analyzing predictive outcomes produced by the HDT versus known cases under various conditions, the modeler can confirm the HDT's representativeness, acceptable accuracy, and robust predictability. If done correctly, the HDT should closely match known scenarios. If not, the HDT parameters can be adjusted until the desired representativeness, accuracy, and predictability are achieved.

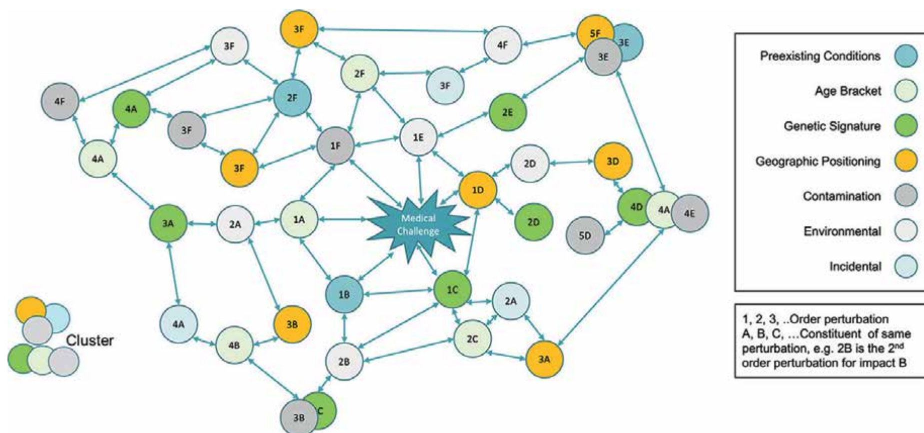

Figure 5.
Example of generic health directed-graph vertices and edges.

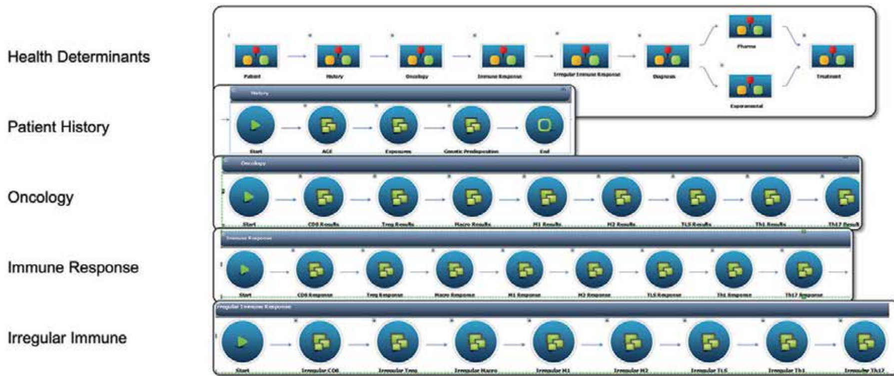

Figure 6.
Example of variable organization and perturbed representation of patient health.

This provides an opportunity to reverse engineer the cause of the discrepancy and ultimately leads to the discovery of new insights or the identification of unknowns by finding missing definitions or introducing new parameters at various levels of the graphs. Such efforts are particularly important once the modeling process has been established since changes in health can occur at any time and necessitate the ability to quickly identify a new risk and apply therapeutic actions.

3.2.4 Step 4: validate findings

To confirm HDT scenario findings and further enhance subject matter expertise, any new predictions or causal relationship insights should be vetted through traditional randomized experiments designed to mimic digital findings in the physical world. Performing targeted experiments to understand failed predictions is a proven method for systematically discovering new epidemiological knowledge [67]. Since model predictions are based on a system reconstruction that represents the totality of what is known about human health, such predictions are a critical test of the current comprehensive understanding of epidemiology for a target patient. Incorrect predictions can be used to discover determinants by classifying them and understanding their underlying causes.

3.2.5 Step 5: certify models

After validating the findings, dynamic patterns encapsulating behaviors, dependencies, and surrounding rules for health behaviors should be stored in a database to support ML and predictive analysis activities. Rules can include remedial options for risk avoidance or preventive interventions. Every human has some biological characteristics that make him or her unique, but in large part, the essential components of health share many commonalities across patients. Building libraries that contain certified dynamic patterns of generic patient models that cover the full hierarchy of genes and biology and various disease states as well as external health influencers, such as behavioral, environmental, and socioeconomic factors, can help provide base models for many HDT applications as well as accelerate model customization tasks in clinical practice. Encouraging contributions to open libraries from related areas of science provides an opportunity to continuously enhance the domain knowledge, support better collaboration across disciplines, and provide greater accessibility to the latest research.

3.2.6 Step 6: use in clinical applications

All combinations of certified generic HDT models and related cognitive findings can be customized with patient-specific data collected from electronic health records and/or medical evaluations to be used in clinical care settings (see **Figure 7**). After completing this customization, care providers or artificial (AI) automated technologies can use the HDT to evaluate scenarios under different patterns of initial conditions and dynamic constraints to identify the circumstances under which health risks increase and use the corresponding information to evaluate the case.

Working to establish universally accepted health metrics would provide clinicians with a framework to quantify morbidity risk and evaluate various intervention options. **Table 1** provides an overview of proposed HDT health scoring metrics.

3.3 Example HDT research use case

The following exploratory multimorbidity use case demonstrates how HDTs can provide a framework to unify NCD research across multiple disciplines. In the following scenario, an HDT is created for a hypothetical patient named Bob Smith. The ledger shows changes in health metrics as the patient's circumstances evolve and new datasets are added, e.g., new test results (see **Figure 8**).

Using the situational data revealed from the HDT, the ultimate objective is to prevent NCDs for individual patients. Scientists can use HDTs to predictively analyze how changing patient parameters may result in a health risk or support a more optimal health outcome (see **Figure 9**).

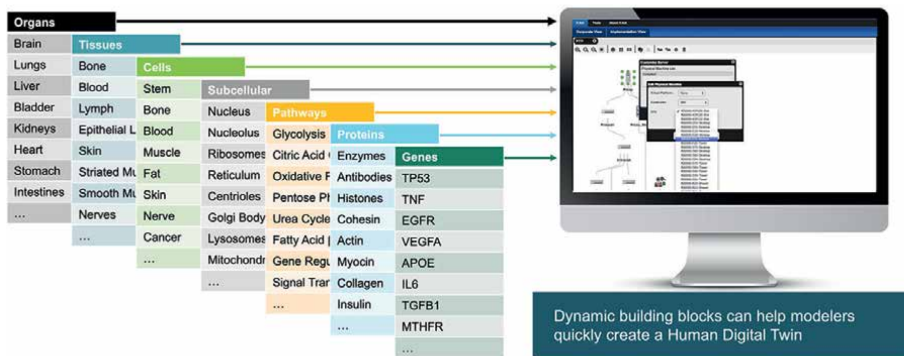

Figure 7.
Libraries of generic models and AI rules provide a starting point for custom patient HDTs in clinical practices.

Human health dependability (HhD) score	Represents the impact of time sensitive interdependencies on human health metrics, which provides an early indication of whether the patient is approaching a health risk.
Energy reserve	Measures the health of patient's immune system and its ability to fight disease.
Mean tolerance score	Measures how quickly the health of the patient may deteriorate over time.

Table 1.
Definition of patient health metrics.

Bob Smith Evolving HDT Ledger	Human Health Dependability		Energy Reserve	mean Tolerance Score
Visit 01-Mar-2018 Diagnosis	98.6		72.4	27.6
Patient History	98.6	Comorbidity	72.4	27.6
Test 03-Mar-2018 Diagnosis	96.7		62.2	3.8
Patient History	98.4		72.2	27.8
Phlebotomy	98.3		86.2	13.8
Visit 04-Mar-2018 Diagnosis	65.4		36.7	15.5
Patient History	92.8	Comorbidity	60.6	39.4
Prostate	70.5		60.6	39.4
Visit 12-Jun-2019 Diagnosis	50.8		22.4	6.1
Patient History	91.3	Multimorbidity	60.3	39.7
Prostate	69.0		60.3	39.7
Phlebotomy	80.7		61.6	38.4
Visit 26-Jun-2019 Diagnosis	32.8		20.7	6.8
CAD	51.9	Multimorbidity	57.0	43.0
Patient History	91.7		60.3	39.7
Prostate	69.0		60.3	39.7
Visit 17-Apr-2020 Diagnosis	7.8		10.5	3.4
Delta	42.2		53.8	46.2
CAD	29.2	Multimorbidity	53.8	46.2
Patient History	91.9		60.3	39.7
Prostate	69.0		60.3	39.7

Figure 8.
HDT ledger for hypothetical patient.

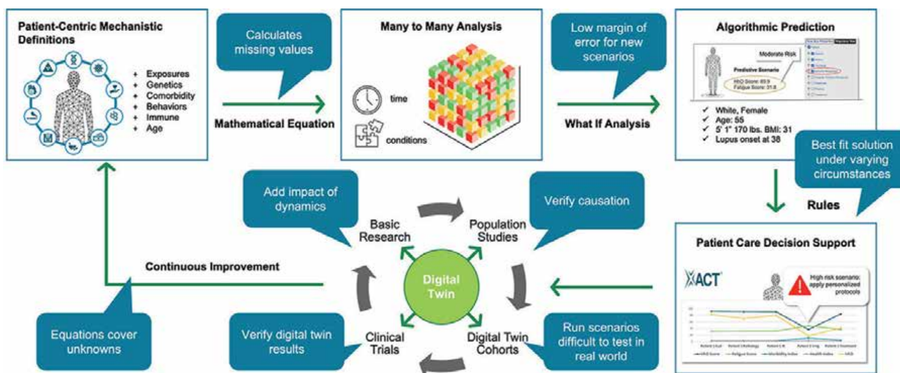

Figure 9.
Use of HDTs for preventive medicine research and personalization of clinical care disease prevention decisions.

In clinical settings, HDT capabilities could rapidly identify a potential problem with immediate analysis of root causes and proposed corrective actions. The active monitoring of HDT outcomes would provide a fully vetted platform to support individualized and proactive patient risk avoidance and suggest personalized preventive protocols when necessary (see **Figure 10**).

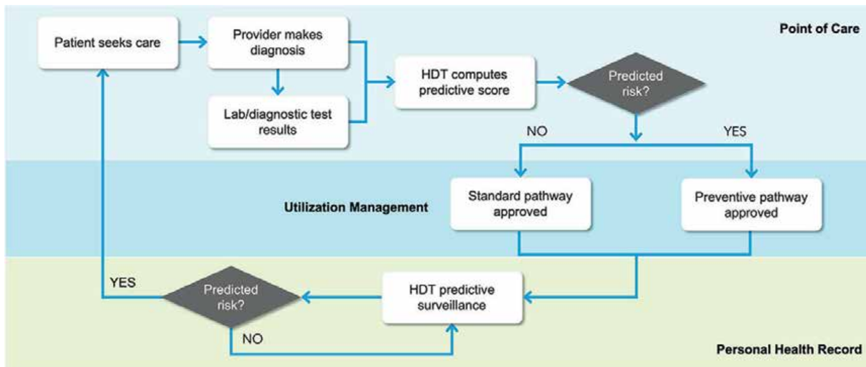

Figure 10.
HDT clinical decision tree.

4. Open challenges and opportunities

A new preventive medicine paradigm that provides a path to contain skyrocketing healthcare costs, extend lifespans, improve citizens' well-being, and increase nations' productivity is a worthy pursuit. Still, obstacles persist.

4.1 HDT ethical challenges

Achieving timely preventive diagnosis, prognosis, intervention, or treatment optimization involves various tasks to build and operate the HDT, including data collection, analysis of current or future health states, and risk scoring. The quality of data inputs and outputs is always a concern. Reliable results cannot be achieved with low-quality data or the wrong analysis methods. Each stage of HDT development and use presents different ethical challenges.

4.1.1 HDT development

Across all industries, establishing credibility and confidence in AI is key to success. Disease prevention and precision medicine require AI to compute all the parameters influencing human health. All HDT vendors should adhere to responsible AI standards to ensure their use for critical applications is safe, trustworthy, and unbiased. The use of AI and ML in treatment or diagnosis must avoid distributional shifts—or else the target data will not match ongoing patient data and will lead to inaccurate conclusions, misjudgment, or incorrect risk scoring. This can be accomplished with modeling methods that are Robust, Explainable, Ethical, and Efficient. Many efforts are underway by technology and research communities to establish responsible AI standards.

HDT developers must use data that reflects the characteristics of the person served by the application and pay extra attention to calibrating and validating algorithms to avoid biased or discriminatory results. Also, it is essential to consider how HDT developers' values and conception of health or diseases may influence decisions regarding which information to present and how to score risks. Any proposed capabilities will need research validation and future collaborations with research centers. All results

should be published in peer-reviewed journals and presented at conferences to help target audiences understand how AI backed with HDT predicts patient outcomes and informs clinical decisions.

4.1.2 HDT data inputs

HDTs are constructed and configured using nonidentifiable and identifiable data. For instance, generic HDTs can be built using de-identified data sets, but to make predictions on a person's health trajectory requires specific identifiable data provided by the patient, the patient's care provider, or imported from the patient's electronic health records (EHR). Potential patient-identifiable data includes medical history, demographics, habits, family history, genetic profile, exposure and incidents, medical encounters, diagnosis, orders and prescriptions, and test results. To avoid violating a person's privacy and autonomy rights, informed consent is always required whenever data is collected or used. Measures should be taken to prevent service providers from secretly collecting more data than necessary and exploiting the data for financial gains [68].

4.1.3 HDT data outputs

HDTs compute 3 categories of synthetic data outputs that can be used for personalized health monitoring, diagnosis, prognosis, prevention, and treatment.

- Descriptive information covers what has happened or is happening to a person's health.

- Predictive information offers foresight into what will likely happen to a person's health.

- Prescriptive information suggests which action or intervention should be taken to improve or restore a person's health.

One of the biggest challenges in the transition to rules-based medicine is the impact on the role of physicians. Any change perceived as decreasing a doctor's control over a patient's fate may not be well received. If AI predicts a certain outcome, doctors and patients want to understand the logic behind the prediction. Therefore, any technology vendor must work with target audiences to define the right product features/benefits and plan a phased rollout of product capabilities that support desired use cases.

Contrary to the goal of empowerment, HDTs might burden patients with a sense of powerlessness, guilt, and anxiety, especially in the case of early lifestyle interventions such as diabetes, hypertension, and obesity [69]. The goal of earlier diagnosis and intervention could lead to overdiagnosis and bodily harm [70]. For example, many bioethicists and clinicians believe genetic testing for BRCA1 and BRCA2 mutations might cause overtreatment [71, 72].

Treating predicted NCD cases and patients with confirmed diseases similarly is morally problematic. Predictive diagnosis labels may increase concern about potential diseases and the desire for more invasive treatments. Evidence suggests that disease labels affect people's psychological responses and healthcare decisions [73]. For example, Nickel et al. [74] suggest removing cancer labels for low-risk conditions may help reduce overdiagnosis and overtreatment.

4.2 Healthcare stakeholder opportunities

The innovative use of HDTs has the potential to align healthcare stakeholders towards a common goal of improving the value of healthcare for all citizens. Innovation and collaboration across healthcare stakeholders can help take HDT from concept to value.

- *Scientists*: HDT will speed precision medicine research by enabling new causal analysis capabilities that were previously impossible. The computational capacity to evaluate the effect of millions of dynamic multiscale variables exists, but readying HDT for clinical use requires that researchers work with mathematicians to develop an accurate representation of the parts that define health and the relationships between these parts. The validation of HDT-derived findings through in vivo studies and traditional data analysis will help translate research into effective precision medicine protocols and build confidence in the approach.

- *Technology providers*: Technology companies are building clinical decision support systems (CDSS) to help clinicians keep up with expanding medical knowledge and incorporate precision medicine concepts into practice. CDSS matches an individual patient's characteristics to a computerized clinical knowledge base and then offers clinicians patient-specific assessments or recommendations for a decision [75]. The objective is admirable, but doctors hesitate to use AI-based CDSS recommendations without transparency. By replacing population averages with the exact mechanisms that define how genes, autoimmune responses, environment, and lifestyle choices influence the health of individual patients, HDT removes variability and randomness. Pairing the determinism of HDT with ML can help technology providers build confidence in HDT-based CDSS solutions.

- *Care providers*: One of the biggest obstacles to HDT rules-based medicine is the impact on the role of physicians. Trust and acceptance of new AI technologies must be established to improve healthcare efficiency. Through scoring metrics that are easy to interpret and explain, HDT can allow care providers to predictively identify at-risk patients and diagnose asymptomatic cases. With HDT, doctors can easily customize and approve recommended treatment plans based on the latest evidence-based medical guidelines and the patient's unique medical characteristics—biomarkers, genes, and other clinical indicators. As new risks form, automated algorithms will help care providers proactively recommend the right actions to patients at the right time.

- *Public health authorities*: Most experts agree that a significant decrease in mortality, leading to improved healthcare economics and survival rates, can only be obtained through earlier diagnosis. The perception that disease control programs are too expensive or complicated significantly reduces the willingness of public health agencies to invest in such services. It is difficult to prove a return on investment for population-based preventive services or justify the anticipated strain on limited healthcare resources. HDT can help eliminate these barriers by providing a more granular way to identify at-risk patients, plan interventions that have the best chance of success, and enforce highly deterministic clinical pathways characterized by a high level of reproducibility of care. By investing in HDT research and explainable AI-enabled CDSS that support non-invasive

asymptomatic disease detection years before conventional diagnosis, countries can measurably reduce national healthcare costs, improve citizen wellbeing, judiciously manage healthcare resources, and ensure equal access to quality care.

- *Regulators*: In many cases, medical device certification requirements, which apply to CDSS as software medical devices, hinder innovation. A collaborative solution between technology providers and regulators will be necessary to modernize regulatory frameworks and accelerate the time to market for AI-based CDSS. Certifying AI rules using outdated, static medical control frameworks is impossible. To translate state-of-the-art precision medicine research and HDT-derived intelligence into clinical value, AI rules must be as dynamic as the health problems they are meant to solve. In response, regulators must expedite the adoption of new risk-based controls or other flexible frameworks that meet market requirements for robust, explainable, ethical, and efficient AI-enabled CDSS.

5. Conclusion

Operationalizing HDT in clinical practice will take strong commitment and collaboration between scientists, doctors, technologists, policymakers, regulators, and payors, but the promised payoffs are worth the pursuit. Collectively, HDT, AI, and CDSS technologies can provide the impetus citizens, governments, payors, and care providers need to finally shift the focus of medicine from sickcare to healthcare.

Healthcare faces an NCD crisis that has not improved despite many technological and biological breakthroughs of the twenty-first century. While some people may push for collecting more health record data, more rigorous diagnosis training programs, or better coordination between healthcare specialists as the solution, none of these actions will improve the standard of patient care or judiciously manage NCD-associated costs if uncertainty persists.

Gaining confidence in any given analysis is the backbone of human and artificial decision-making. The uncertainty inherent in probability-based NCD risk stratification must be solved, or any attempts to meet the goals of personalized precision medicine will remain prone to error, especially in multimorbidity cases, because complex and ambiguous presentations cannot be covered by experience and research data alone. No matter how much data is collected or how well-trained a doctor may be, the dynamics of human health cannot always be inferred from knowledge, experience, or intuition.

On the surface, the call to upend hundreds of years of preventive practices built upon analogies and statistics may seem radical, but the disruption is necessary. Research has proven that complex interactions between genetics, biochemistry, physiology, microbiology, and biomechanics affect health and indirectly impact less obvious factors, like extrinsic factors such as public policy, environment, or social interactions with others. To improve NCD control, new methods are needed to relate these complex causes quickly and reliably into effects for an individual patient.

Now is the time to advance preventive care decisions from an art form to a scientific discipline that takes advantage of the deterministic modeling methods used in other branches of science. Pairing mechanistic HDTs with robotics and AI capabilities will allow clinicians to autonomously predict patient outcomes without a modeling expert's assistance. To this end, scientists, researchers, and practitioners should

collaborate to prove the robustness of the solution in prospective research cases and build the interfaces necessary to support the use of HDT in critical preventive medicine use cases.

Conflict of interest

While the author did not receive, at any time, any payment or services from a third party for any aspect of the submitted work, he wishes to declare the use of a proprietary algorithm and methodologies, which are subject to a pending patent.

Author details

Nabil Abu el Ata
URM Forum, Cannes, France

*Address all correspondence to: nabil@urmgrp.com

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References

[1] World Health Organization. Non-Communicable Diseases [Internet]. 2022. Available from: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases [Accessed: September 12, 2023]

[2] NCD Alliance. NCDs [Internet]. 2023. Available from: https://ncdalliance.org/why-ncds/NCDs [Accessed: September 12, 2023]

[3] World Health Organization, editors. Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013-2020 [Internet]. 2013. Available from: https://www.who.int/publications/i/item/9789241506236 [Accessed: September 12, 2023]

[4] Bojola F, Taye W, Samuel H, Mulatu B, Kawza A, Mekuria A. Non-communicable diseases (NCDs) and vulnerability to COVID-19: The case of adult patients with hypertension or diabetes mellitus in Gamo, Gofa, and south Omo zones in southern Ethiopia. PLoS One. 2022;**17**(1):e0262642. DOI: 10.1371/journal.pone.0262642

[5] Pan American Health Organization. Economics of NCDs [Internet]. 2023. Available from: https://www.paho.org/en/topics/economics-ncds [Accessed: September 12, 2023]

[6] Garg CC, Evans DB. What Is the Impact of Noncommunicable Diseases on National Health Expenditures: A Synthesis of Available Data. Geneva: World Health Organization [Internet]; 2011. Available from: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=1e37be207a3d68ed2ba3d68fe47439fe2a4b57d1 [Accessed: September 10, 2023]

[7] Muka T, Imo D, Jaspers L, Colpani V, Chaker L, van der Lee SJ, et al. The global impact of non-communicable diseases on healthcare spending and national income: A systematic review. European Journal of Epidemiology. 2015;**30**(4):251-277. DOI: 10.1007/s10654-014-9984-2

[8] Mariotto AB, Enewold L, Zhao J, Zeruto CA, Yabroff KR. Medical care costs associated with cancer survivorship in the United States. Cancer Epidemiology, Biomarkers & Prevention. 2020;**29**:1304-1312. DOI: 10.1158/1055-9965.EPI-19-1534

[9] National Health Expenditure Data: Historical. Center for Medicare & Medicaid Services [Internet]. 2021. Available from: https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/NationalHealthExpendData/NationalHealthAccountsHistorical [Accessed: September 12, 2023]

[10] Health System Tracker. What Do We Know About Spending Related to Public Health in the U.S. and Comparable Countries? [Internet]. 2020. Available from: https://www.healthsystemtracker.org/chart-collection/what-do-we-know-about-spending-related-to-public-health-in-the-u-s-and-comparable-countries/ [Accessed: September 12, 2023]

[11] World Health Organization. Early Cancer Diagnosis Saves Lives, Cuts Treatment Costs [Internet]. 2017. Available from: https://www.who.int/news/item/03-02-2017-early-cancer-diagnosis-saves-lives-cuts-treatment-costs [Accessed: September 12, 2023]

[12] Ioannidis JPA. Why most published research findings are false. PLoS Medicine. 2005;**2**:e124. DOI: 10.1371/journal.pmed.0020124

[13] National Cancer Institute. Crunching Numbers: What Cancer Screening Statistics Really Tell Us [Internet]. 2018. Available from: https://www.cancer.gov/about-cancer/screening/research/what-screening-statistics-mean [Accessed: September 12, 2023]

[14] Keating NL, Pace LE. Breast cancer screening in 2018: Time for shared decision making. Journal of the American Medical Association. 2018;**319**(17):1814-1815. DOI: 10.1001/jama.2018.3388

[15] Grossman DC, Curry SJ, Owens DK, Bibbins-Domingo K, Caughey AB, Davidson KW, et al. Screening for prostate cancer: US preventive services task force recommendation statement. Journal of the American Medical Association. 2018;**319**(18):1901-1913. DOI: 10.1001/jama.2018.3710

[16] Auffray C, Noble D, Nottale L, et al. Progress in integrative systems biology, physiology and medicine: Towards a scale-relative biology. European Physical Journal A: Hadrons and Nuclei. 2020;**56**:88. DOI: 10.1140/epja/s10050-020-00090-3

[17] Maziak W. Is uncertainty in complex disease epidemiology resolvable? Emerging Themes in Epidemiology. 2015;**12**:7. DOI: 10.1186/s12982-015-0028-5

[18] Pybus O, Rambaut A. Evolutionary analysis of the dynamics of viral infectious disease. Nature Reviews. Genetics. 2009;**10**:540-550. DOI: 10.1038/nrg2583

[19] Ahn AC, Tewari M, Poon C-S, Phillips RS. The limits of reductionism in medicine: Could systems biology offer an alternative? PLoS Medicine. 2006;**3**(6):e208. DOI: 10.1371/journal.pmed.0030208

[20] Sweeney K, Kernick D. Clinical evaluation: Constructing a new model for postnormal medicine. Journal of Evaluation in Clinical Practice. 2002;**8**:131-138. DOI: 10.1046/j.1365-2753.2002.00312.x

[21] Prokop A, Csukas B, editors. Systems Biology—Integrative Biology and Simulation Tools. Dordrecht: Springer; 2013. DOI: 10.1007/978-94-007-6803-1

[22] Capra F, Luisi PL. The Systems View of Life—A Unifying Vision. Cambridge: Cambridge University Press; 2014. DOI: 10.1017/CBO9780511895555

[23] Abu el Ata N. Modeling the Spatiotemporal Evolution of Nonlinear Systems Using a Perturbed Graph Solution [Internet]. 2022. Available at: https://urmforum.org/wp-content/uploads/NAbuelata-Perturbed-Graph-Solution.pdf [Accessed: September 12, 2023]

[24] van Oostrom SH, Picavet HS, van Gelder BM, Lemmens LC, Hoeymans N, van Dijk CE, et al. Multimorbidity and comorbidity in the Dutch population-data from general practices. BMC Public Health. 2012;**12**:715. DOI: 10.1186/1471-2458-12-715

[25] Conrad M. What is the use of chaos? In: Holden A, editor. Chaos. Princeton: Princeton University Press; 1986. pp. 3-14. DOI: 10.1515/9781400858156.3

[26] Buchman TG. The community of self. Nature. 2002;**420**:246-251. DOI: 10.1038/nature01260

[27] Scheer FA, Czeisler CA. Melatonin, sleep, and circadian rhythms. Sleep Medicine. 2005;**9**:5-9. DOI: 10.1016/j.smrv.2004.11.004

[28] Poon CS, Merrill CK. Decrease of cardiac chaos in congestive heart

failure. Nature. 1997;**389**:492-495. DOI: 10.1038/39043

[29] Goldberger A, Amaral L, Hausdorff J, Ivanov P, Peng C. Fractal dynamics in physiology: Alterations with disease and aging. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**(Suppl):12466-12472. DOI: 10.1073/pnas.012579499

[30] Goldberger A. Non-linear dynamics for clinicians: Chaos theory, fractals, and complexity at the bedside. Lancet. 1996;**347**:1312-1314. DOI: 10.1016/s0140-6736(96)90948-4

[31] Abu el Ata N, Schmandt R. The Tyranny of Uncertainty. Berlin, Heidelberg: Springer; 2016. DOI: 10.5555/2961887

[32] Abu el Ata N, Drucbert A. Leading from under the Sword of Damocles. Berlin, Heidelberg: Springer; 2017. DOI: 10.1007/978-3-662-56300-7

[33] Zaloga G. Hypocalcemia in critically ill patients. Critical Care Medicine. 1992;**20**:251-262. DOI: 10.1097/00003246-199202000-00014

[34] Zaloga G, Sager A, Black K, Prielipp R. Low dose calcium administration increases mortality during septic peritonitis in rates. Circulatory Shock. 1992;**37**:226-229. PMID: 1423913

[35] Oliveira-Filho J, Silva S, Trabuco C, Pedreira B, Sousa E, et al. Detrimental effect of blood pressure reduction in the first 24 hours of acute stroke onset. Neurology. 2003;**61**:1047-1051. DOI: 10.1212/01.WNL.0000092498.75010.57

[36] Degn H, Holden AV, Olsen LF, editors. Chaos in Biological Systems. New York: Springer; 1987. DOI: 10.1007/978-1-4757-9631-5

[37] Guruprasad L. Human SARS CoV-2 spike protein mutations. Proteins. 2021;**89**(5):569-576. DOI: 10.1002/prot.26042

[38] Adjiri A. DNA mutations may not Be the cause of cancer. Oncology and Therapy. 2017;**5**(1):85-101. DOI: 10.1007/s40487-017-0047-1

[39] Barabási AL, Oltvai ZN. Network biology: Understanding the cell's functional organization. Nature Reviews. Genetics. 2004;**5**:101-113. DOI: 10.1038/nrg1272

[40] Barabási AL, Gulbahce N, Loscalzo J. Network medicine: A network-based approach to human disease. Nature Reviews. Genetics. 2011;**12**:56-58. DOI: 10.1038/nrg2918

[41] Baker RE, Peña JM, Jayamohan J, Jérusalem A. Mechanistic models versus machine learning, a fight worth fighting for the biological community? Biology Letters. 2018;**14**:20170660. DOI: 10.1098/rsbl.2017.0660

[42] Abu el Ata N. Analytical Solution the Planetary Perturbation on the Moon [Thesis]. Paris, France: Sorbonne School of Mathematical Sciences; 1978

[43] Chapront J, Abu el Ata N. Analytical development of the inverse of the distance. Astronomy and Astrophysics. 1975;**38**(1):57-66. . In French. DOI: 10.1007/BF01228813

[44] Chapront J, Abu el Ata N. Planetary perturbations of the moon in elliptic variables. Astronomy and Astrophysics. 1977;**55**:38-94. In French

[45] Qian Y, Wan H, Yang B, Golaz JC, Harrop B, Hou Z, et al. Parametric

sensitivity and uncertainty quantification in the version 1 of E3SM atmosphere model based on short perturbed parameter ensemble simulations. Journal of Geophysical Research: Atmospheres. 2018;**123**:13046-13073. DOI: 10.1029/2018JD028927

[46] Morley SK, Welling DT, Woodroffe JR. Perturbed input ensemble modeling with the space weather modeling framework. Space Weather. 2018;**16**:1330-1347. DOI: 10.1029/2018SW002000

[47] Bissell MJ, Labarge MA. Context, tissue plasticity, and cancer: Are tumor stem cells also regulated by the microenvironment? Cancer Cell. 2005;**7**(1):17-23. DOI: 10.1016/j.ccr.2004.12.013

[48] Lehmann PV et al. Determinant spreading and the dynamics of the autoimmune T-cell repertoire. Immunology Today. 1993;**14**(5):203-208. DOI: 10.1016/0167-5699(93)90163-F

[49] Lusis A, Attie A, Reue K. Metabolic syndrome: From epidemiology to systems biology. Nature Reviews. Genetics. 2008;**9**:819-830. DOI: 10.1038/nrg2468

[50] Skead K, Ang Houle A, Abelson S, et al. Interacting evolutionary pressures drive mutation dynamics and health outcomes in aging blood. Nature Communications. 2021;**12**(4921):8-9. DOI: 10.1038/s41467-021-25172-8

[51] Stalidzan E, Zanin M, Tieri P, Castiglione F, Polster A, Scheiner S, et al. Mechanistic modeling and multiscale applications for precision medicine: Theory and practice. Network and Systems Medicine. 2020;**3**(1):36-56. DOI: 10.1089/nsm.2020.0002

[52] Kuepfer L, Schuppert A. Systems medicine in pharmaceutical research and development. Methods in Molecular Biology. 2016;**1386**:87-104. DOI: 10.1007/978-1-4939-3283-2_6

[53] Stéphanou A, Fanchon E, Innominato PF, et al. Systems biology, systems medicine, systems pharmacology: The what and the why. Acta Biotheoretica. 2018;**66**:345-365. DOI: 10.1007/s10441-018-9330-2

[54] Wang Y, Yuan Y, Ma Y, et al. Time-dependent graphs: Definitions, applications, and algorithms. Data Science and Engineering. 2019;**4**:352-366. DOI: 10.1007/s41019-019-00105-0

[55] Przytycka TM, Singh M, Slonim DK. Toward the dynamic interactome: It's about time. Briefings in Bioinformatics. 2010;**11**(1):15-29. DOI: 10.1093/bib/bbp057

[56] Marchetti-Bowick M, Yin J, Howrylak JA, Xing EP. A time-varying group sparse additive model for genome-wide association studies of dynamic complex traits. Bioinformatics. 2016;**32**(19):2903-2910. DOI: 10.1093/bioinformatics/btw347

[57] Rao A, Hero AO III, Engel JD, et al. Inferring time-varying network topologies from gene expression data. EURASIP Journal on Bioinformatics and Systems Biology. 2007;**2007**(1):51947. DOI: 10.1155/2007/51947

[58] Han JDJ, Bertin N, Hao T, Goldberg DS, Berriz GF, Zhang LV, et al. Evidence for dynamically organized modularity in the yeast protein–protein interaction network. Nature. 2014;**430**(6995):88. DOI: 10.1038/nature02555

[59] Lebre S, Becq J, Devaux F, Stumpf MP, Lelandais G. Statistical inference of the time-varying structure of gene-regulation networks. BMC

Systems Biology. 2010;**4**(1):130. DOI: 10.1186/1752-0509-4-130

[60] Garthwaite K, Holdridge DB, Mulholland JD. A preliminary special perturbation theory for the lunar motion. The Astronomical Journal. 1970;**75**:1133-1139. Available from: https://adsabs.harvard.edu/full/1970AJ.....75.1133G [Accessed: September 12, 2023]

[61] Bruynseels K, Santoni de Sio F, van den Hoven J. Digital twins in health care: Ethical implications of an emerging engineering paradigm. Frontiers in Genetics. 2018;**9**:31. DOI: 10.3389/fgene.2018.00031

[62] Abu el Ata N, et al. Covid-19: Worldwide Viral Infection Model [Internet]. 2020. Available from: https://urmforum.org/portfolio-item/covid-19-worldwide-viral-infection-model/ [Accessed: September 12, 2023]

[63] Azimi P, Mohammadi HR. Predicting endoscopic third ventriculostomy success in childhood hydrocephalus: An artificial neural network analysis. Journal of Neurosurgery: Pediatrics. 2014;**13**:426-432. DOI: 10.3171/2013.12.PEDS13423

[64] Fayyad UM, Piatetsky-Shapiro G, Smyth P, editors. From data mining to knowledge discovery: An overview. In: Advances in Knowledge Discovery and Data Mining. Vol. 21. Menlo Park, CA: AAAI Press; 1996. pp. 1-34. DOI: doi.org/10.1609/aimag.v17i3.1230

[65] Abu el Ata N. Predictive deconstruction of dynamic complexity. U.S. Provisional Application No. 61/438,045; 2011

[66] Abu el Ata N, Perks MJ. Solving the Dynamic Complexity Dilemma. Berlin, Heidelberg: Springer; 2014. pp. 143-164. DOI: 10.1007/978-3-642-54310-4

[67] Orth JD, Palsson BØ. Systematizing the generation of missing metabolic knowledge. Biotechnology and Bioengineering. 2010;**107**:403-412. DOI: 10.1002/bit.22844

[68] Prainsack B. Personalized Medicine: Empowered Patients in the 21st Century? 1st ed. New York: NYU Press; 2017. DOI: doi.org/10.18574/nyu/9781479814879.001.0001

[69] Marantz PR. Blaming the victim: The negative consequence of preventive medicine. American Journal of Public Health. 1990;**80**(10):1186-1187. DOI: 10.2105/ajph.80.10.1186

[70] Huang PH, Kim KH, Schermer M. Ethical issues of digital twins for personalized health care service: Preliminary mapping study. Journal of Medical Internet Research. 2022;**24**(1):e33081. DOI: 10.2196/33081

[71] U.S. Preventive Services Task Force. Genetic risk assessment and BRCA mutation testing for breast and ovarian cancer susceptibility: Recommendation statement. Annals of Internal Medicine. 2005;**143**(5):355-361. DOI: 10.7326/0003-4819-143-5-200509060-00011

[72] Katz SJ, Morrow M. Addressing overtreatment in breast cancer: The doctors' dilemma. Cancer. 2013;**119**(20):3584-3588. DOI: 10.1002/cncr.28260

[73] Garand L, Lingler JH, Conner KO, Dew MA. Diagnostic labels, stigma, and participation in research related to dementia and mild cognitive impairment. Research in Gerontological Nursing. 2009;**2**(2):112-121. DOI: 10.3928/19404921-20090401-04

[74] Nickel B, Moynihan R, Barratt A, Brito JP, McCaffery K. Renaming low

risk conditions labelled as cancer. BMJ. 2018;**362**:k3322. DOI: 10.1136/bmj.k3322

[75] Sim I et al. Clinical decision support systems for the practice of evidence-based medicine. Journal of the American Medical Informatics Association. 2001;**8**:527-534. DOI: 10.1136/jamia.2001.0080527

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This book presents the new advances and new perspectives of personalized medicine, emphasizing personalized, predictive medicine, personalized preventive medicine, personalized therapy, and personalized diagnosis/prognostic assessment. The research and practice of multiomics-based pattern biomarkers will significantly contribute to personalized medicine, including deeply addressing the accurate molecular mechanism, stratifying the patients, discovering effective biomarkers, and determining the effective therapeutic targets/drugs for patients to significantly benefit the patients and their families to reduce the medical cost and improve the life quality of patients.

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