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Precision medicine: Recent advances, current challenges and future perspectives

Edited by
Oriana Awwad, Mamoun Ahram, Mariam Abdel Jalil and Francesca Coperchini

Published in
Frontiers in Pharmacology
Frontiers in Genetics

ISSN 1664-8714
ISBN 978-2-8325-5042-7
DOI 10.3389/978-2-8325-5042-7

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Precision medicine: Recent advances, current challenges and future perspectives

Topic editors

Oriana Awwad — The University of Jordan, Jordan
Mamoun Ahram — The University of Jordan, Jordan
Mariam Abdel Jalil — The University of Jordan, Jordan
Francesca Coperchini — University of Pavia, Italy

Citation

Awwad, O., Ahram, M., Jalil, M. A., Coperchini, F., eds. (2024). *Precision medicine: Recent advances, current challenges and future perspectives*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5042-7

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frontiers | Frontiers in Pharmacology

TYPE Editorial
PUBLISHED 07 June 2024
DOI 10.3389/fphar.2024.1439276

OPEN ACCESS

EDITED AND REVIEWED BY
José A. G. Agúndez,
University of Extremadura, Spain

*CORRESPONDENCE
Oriana Awwad,
o.awwad@ju.edu.jo

RECEIVED 27 May 2024
ACCEPTED 30 May 2024
PUBLISHED 07 June 2024

CITATION
Awwad O, Ahram M, Coperchini F and Jalil MA (2024), Editorial: Precision medicine: recent advances, current challenges and future perspectives.
Front. Pharmacol. 15:1439276.
doi: 10.3389/fphar.2024.1439276

Editorial: Precision medicine: recent advances, current challenges and future perspectives

Oriana Awwad[1]*, Mamoun Ahram[2], Francesca Coperchini[3] and Mariam Abdel Jalil[1]

[1]Department of Biopharmaceutics and Clinical Pharmacy, School of Pharmacy, The University of Jordan, Amman, Jordan, [2]Department of Physiology and Biochemistry, School of Medicine, The University of Jordan, Amman, Jordan, [3]Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy

KEYWORDS

precision medicine, personalized medicine, pharmacogenetics, gene variants, pharmacologic targets, genotyping

Editorial on the Research Topic
Precision medicine: recent advances, current challenges and future perspectives

Unlike most medical treatments obtained from large-scale studies and designed as "one-size-fits-all" approach, precision medicine is a modern concept of personalized treatment that aims to provide patients with tailored medical interventions to each individual characteristics (Beckmann and Lew, 2016; Akhoon, 2021; Marques et al., 2024). Precision medicine is advantageous in being dynamic, evolving, and wholesome as it accounts for patients' medical history, molecular profiles, lifestyle, and external factors within their environment (Faulkner et al., 2020; Denny and Collins, 2021). This approach is designed to facilitate enhanced screening, earlier disease detection, more precise diagnosis, and targeted treatment, allowing patients to receive therapies that work best for them for a more effective and safer clinical management and a more efficient healthcare system (Beckmann and Lew, 2016; Faulkner et al., 2020; Akhoon, 2021; Wang and Wang, 2023).

This patient-centered clinical approach needs to identify specific disease subtypes and to classify patients into various subpopulations that differ in their response and susceptibility to traditional treatments (Wang and Wang, 2023; Marques et al., 2024). The diversity of pharmacological mechanisms and targets can permit therapies to efficiently treat particular clinical patients.

Despite the potential of precision medicine and the growing number of customized drugs, there is a need for evidence of the clinical value of integrating such treatments into healthcare systems. Efforts are also needed to determine the best medical intervention for each patient and medical condition. This Research Topic aims to tackle these issues.

The review by Tavazzani et al. identifies the major challenges in diagnosing and treating inner ear diseases. These include access to the inner ear and difficulty in sampling inner ear fluids. The authors then discuss the possible roles of innovative technologies to overcome such challenges. Combining microneedles, nanocarriers, and gene therapy holds great

potential in revolutionizing and personalizing the treatment of inner ear diseases of different etiologies.

Biswas et al. reviewed drugs used in autism spectrum disorder (ASD), focusing on their pharmacokinetics, pharmacodynamics, and genetic and non-genetic factors affecting efficacy and safety. They found that *CYP2D6* and *DRD2* gene variants were associated with an increased risk of hyperprolactinemia in children taking risperidone. The review also discusses the interactions of ASD drugs with other drugs, such as enzyme inducers or inhibitors, and their effects on drug safety and efficacy. In addition, factors that can hinder the implementation of pharmacogenetics are highlighted.

Biswas et al. also introduce the pharmacogenetics and repurposing of COVID-19 therapies. The authors call to undertake a pharmacogenetic assessment of some drugs, particularly those that target certain CYP/transporters, for a safer, more effective management of COVID-19. The authors also promote the application of computational studies to discover new medications.

Dakilah et al. identify CDC25 phosphatases as promising candidates for therapeutic intervention of cancer due to their biological role in activating cyclin-dependent kinases and, hence, regulating the cell cycle. The review summarizes the evidence related to the dysregulation of CDC25 phosphatases in various types of cancer. In addition, the authors offer an overview of the enzyme genetic variants underlying the importance of CDC25 inhibition as a therapeutic target for individualized cancer treatment.

Shubbar et al. present *CYP2C19*, a gene involved in the metabolism of commonly prescribed medications, as an interesting target for optimizing healthcare provision through precision medicine. The authors demonstrate how genetic variations of *CYP2C19* can play a role in the metabolism of certain drugs affecting the disease-related therapeutic outcomes. The review then addresses *CYP2C19* genotyping as an opportunity to promote drug efficacy and safety with clinical significance in various medical conditions.

Birla et al. describe the principle of polypills, explore their evidence-based strengths and weaknesses in the management of cardiovascular medicine, elaborate on their potential use in the prevention and treatment of cardiovascular diseases, and list relevant clinical trials involving polypills. The review found that polypills can reduce major adverse cardiovascular events, improve medication adherence, and lower healthcare costs. However, challenges include dosage adjustment, acceptability, and the need for more safety studies. Further research is required to assess customizing polypills for personalized therapeutics.

Greco et al. report drug repositioning and its importance in treating thyroid cancer. The authors offer an overview of the anti-diabetic drugs and their anticancer activities. An example is metformin. In drug repositioning, understanding the disease-drug relationship is crucial. This involves using various approaches to discover disease-gene, disease-disease, and disease-target relationships.

The paper by Cavalloro et al. identifies novel multi-target ligands for managing neurodegenerative diseases using a combination of computational and experimental methods. The ligands influence the HuD/brain-derived neurotrophic factor (BDNF) pathway and activate the ubiquitin-proteasome system via two synergistically acting mechanisms. The outcome of this study promotes precision medicine as a powerful tool for managing neurodegenerative diseases. Yet, future research is needed to overcome the related challenges.

As an attempt to integrate genomics into community health, David et al. report the evolution, strategy, and implementation of the NorthShore University HealthSystem, which is an integrated, personalized healthcare delivery system that involves several hospitals and multispecialty group practice of over 140 locations. The system required the development of screening tools, integrated pharmacogenomics programming, educational programming, electronic medical record integration, and robust clinical decision supportive tools. Over 100 primary care providers were trained in genomic medicine, and more than 225,000 patients were screened for hereditary conditions. Over 4,000 patients have been identified to have genetic variations with medical management implications. The successful experience of NorthShore can be applied to other communities.

These studies highlight the importance of collective knowledge of the diverse pharmacological strategies, mechanisms, and targets as well as the in-and-out details of patients for the success of treatments. Such integration may also ultimately lead to changes in the regulatory, ethical, and policy domains of drug therapy (Faulkner et al., 2020; Marques et al., 2024).

Author contributions

OA: Conceptualization, Investigation, Methodology, Project administration, Writing–original draft, Writing–review and editing. MAh: Conceptualization, Investigation, Methodology, Writing–review and editing. FC: Conceptualization, Investigation, Methodology, Writing–review and editing. MAb: Conceptualization, Investigation, Writing–original draft, Methodology.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers in Pharmacology

REVIEW
published: 18 February 2022
doi: 10.3389/fphar.2022.835136

Pharmacogenetics and Precision Medicine Approaches for the Improvement of COVID-19 Therapies

Mohitosh Biswas[1,2,3], *Nares Sawajan*[1,2,4], *Thanyada Rungrotmongkol*[5,6], *Kamonpan Sanachai*[5], *Maliheh Ershadian*[1,2] *and Chonlaphat Sukasem*[1,2,7,8]*

[1]*Division of Pharmacogenomics and Personalized Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand,* [2]*Laboratory for Pharmacogenomics, Somdech Phra Debaratana Medical Center (SDMC), Ramathibodi Hospital, Bangkok, Thailand,* [3]*Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh,* [4]*Department of Pathology, School of Medicine, Mae Fah Luang University, Chiang Rai, Thailand,* [5]*Structural and Computational Biology Research Unit, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok, Thailand,* [6]*Program in Bioinformatics and Computational Biology, Graduate School, Chulalongkorn University, Bangkok, Thailand,* [7]*Pharmacogenomics and Precision Medicine, The Preventive Genomics and Family Check-up Services Center, Bumrungrad International Hospital, Bangkok, Thailand,* [8]*MRC Centre for Drug Safety Science, Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool, United Kingdom*

OPEN ACCESS

Edited by:
Giosuè Costa,
University of Catanzaro, Italy

Reviewed by:
Dmitry A. Sychev,
Russian Medical Academy of Continuous Professional Education, Russia
Isabella Romeo,
University of Catanzaro, Italy

***Correspondence:**
Chonlaphat Sukasem
chonlaphat.suk@mahidol.ac.th

Specialty section:
This article was submitted to Pharmacogenetics and Pharmacogenomics, a section of the journal Frontiers in Pharmacology

Received: *15 December 2021*
Accepted: *24 January 2022*
Published: *18 February 2022*

Citation:
Biswas M, Sawajan N, Rungrotmongkol T, Sanachai K, Ershadian M and Sukasem C (2022) Pharmacogenetics and Precision Medicine Approaches for the Improvement of COVID-19 Therapies. Front. Pharmacol. 13:835136. doi: 10.3389/fphar.2022.835136

Many drugs are being administered to tackle coronavirus disease 2019 (COVID-19) pandemic situations without establishing clinical effectiveness or tailoring safety. A repurposing strategy might be more effective and successful if pharmacogenetic interventions are being considered in future clinical studies/trials. Although it is very unlikely that there are almost no pharmacogenetic data for COVID-19 drugs, however, from inferring the pharmacokinetic (PK)/pharmacodynamic(PD) properties and some pharmacogenetic evidence in other diseases/clinical conditions, it is highly likely that pharmacogenetic associations are also feasible in at least some COVID-19 drugs. We strongly mandate to undertake a pharmacogenetic assessment for at least these drug–gene pairs (atazanavir–*UGT1A1*, *ABCB1*, *SLCO1B1*, *APOA5*; efavirenz–*CYP2B6*; nevirapine–*HLA*, *CYP2B6*, *ABCB1*; lopinavir–*SLCO1B3*, *ABCC2*; ribavirin–*SLC28A2*; tocilizumab–*FCGR3A*; ivermectin–*ABCB1*; oseltamivir–*CES1*, *ABCB1*; clopidogrel–*CYP2C19*, *ABCB1*, warfarin–*CYP2C9*, *VKORC1*; non-steroidal anti-inflammatory drugs (NSAIDs)–*CYP2C9*) in COVID-19 patients for advancing precision medicine. Molecular docking and computational studies are promising to achieve new therapeutics against SARS-CoV-2 infection. The current situation in the discovery of anti-SARS-CoV-2 agents at four important targets from *in silico* studies has been described and summarized in this review. Although natural occurring compounds from different herbs against SARS-CoV-2 infection are favorable, however, accurate experimental investigation of these compounds is warranted to provide insightful information. Moreover, clinical considerations of drug–drug interactions (DDIs) and drug–herb interactions (DHIs) of the existing repurposed drugs along with pharmacogenetic (e.g., efavirenz and *CYP2B6*) and herbogenetic (e.g., andrographolide and *CYP2C9*) interventions, collectively called multifactorial drug–gene interactions (DGIs), may further accelerate the development of precision COVID-19 therapies in the real-world clinical settings.

Keywords: COVID-19, pathogenesis and severity, repurposed drugs, pharmacogenetics, molecular docking, drug-drug interactions, drug-herb interactions, precision medicine

1 INTRODUCTION FOR PHARMACOGENETICS OF COVID-19 TREATMENT

At the end of 2019, a novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started as an emerging pathogen for humans, first appeared in Wuhan, China, in December 2019. This novel virus causes coronavirus disease 2019 (COVID-19), named by the WHO on 11 February 2020, and it has been characterized as a pandemic on 11 March 2020. COVID-19 has become the leading cause of death globally, resulting in huge economic and social disruption internationally (Hodgson et al., 2021). As of 10 September 2021 as declared by the WHO, over 223 million confirmed cases of SARS-CoV-2 infection have been detected globally in which ~4.6 million deaths occurred (WHO, 2021). It is alarming that still now considerably a large number of patients are dying due to COVID-19. The existence of this pandemic virus had been confirmed in over 200 countries or territories, indicating that corona virus was exponentially spread out throughout the world.

One of the leading causes of morbidity and mortality might be the adverse drug reactions (ADRs) associated with current medications administered for the management of COVID-19 since the mortality rate was significantly higher in COVID-19 patients with multiple comorbidities and particularly in older patients (Manjaly Thomas et al., 2020; Ramírez et al., 2020; Biswas et al., 2021a; Falcão et al., 2021; Melo et al., 2021; Rezaee et al., 2021). Polypharmacy is highly predictable in multiple comorbid patients, and also, age-related degradation of organ function in older patients is placing them highly vulnerable to drug–drug interactions (DDIs) and consequently the most notorious ADRs or toxicities of the COVID-19 therapeutics. A recent pharmacovigilance study conducted in Spain reported the 4.75-fold higher incidence of severe ADRs in the COVID-19 patients compared to non-COVID-19 patients, in which the prevalence of severe ADRs was the highest with tocilizumab (59.8%) followed by dexketoprofen (13.9%), azithromycin (8.4%), dexamethasone (7.6%), lopinavir–ritonavir (7.4%), and chloroquine (CQ)/hydroxychloroquine (HCQ) (6.9%) (Ramírez et al., 2020). Another pharmacovigilance study conducted in Brazil with 402 COVID-19 patients indicated that chloroquine (CQ) (OR = 5.4; 95% CI: 1.9–15.6) and HCQ (OR = 2.1; 95% CI: 1.2–3.6) were the only culprit drugs associated with severe ADRs (Melo et al., 2021). A prospective observational study identified a total of 102 ADRs in 149 COVID-19 patients where the incidence of ADRs was significantly higher in patients who have taken HCQ than in the patients who have taken remdesivir (RDV) (47.5 vs. 12.5%; $p < 0.001$), as evidenced recently (Falcão et al., 2021). This is consistent with a predictive study showing that at least 329 DDIs are feasible in patients taking HCQ, and at the very least, 29 severe DDIs were identified from different reputed international interaction resources, predicted to cause severe toxicity of HCQ (Biswas and Roy, 2021). A hospital-based pharmacovigilance study conducted in China identified ~38% ADRs in COVID-19 patients, where drug-induced gastrointestinal disorders were 23% and liver system disorders were ~14%. These ADRs were mainly associated with the use of lopinavir/ritonavir (~64%) and umifenovir (~18%). Multivariate logistic analysis indicated that the number of drugs used while COVID-19 patients were staying in the hospital was one of the strongest independent risk factors for these ADRs (OR: 3.17; 95% CI 1.60–6.27; $p = 0.001$), as reported in this observational study (Sun et al., 2020).

Although there are no specific therapeutic recommendations for treating COVID-19, however, many off-label drugs are being currently administered for the management of COVID-19 and severe ADRs; for example, QT prolongation, cardiac arrhythmias, thrombosis, retinopathy, hepatotoxicity, and increased risk of infection due to DDIs are feasible in these patients as evidenced and suggested elsewhere (Biswas et al., 2020a; Lemaitre et al., 2020; Manjaly Thomas et al., 2020; Ramírez et al., 2020; Sun et al., 2020; Biswas and Roy, 2021; Falcão et al., 2021; Melo et al., 2021; Rezaee et al., 2021).

While it is increasingly true of DDIs for COVID-19 therapeutics, it is likely that the cytochrome P450 (CYP) enzymes or transporter proteins affecting the pharmacokinetics (PK) or pharmacodynamic (PD) properties were mostly involved in the reported and predicted DDIs of drugs used in the treatment of COVID-19. However, the genetic variants modulating the PK/PD profiles of COVID-19 drugs regulating the safety or effectiveness are not clinically studied yet, posing serious scarcity of pharmacogenomic data in the literature.

The PK and PD properties of the COVID-19 drugs are very potential factors to explore the pharmacogenetics association study; however, it appears that drug-developing authorities and scientists did not consider the pharmacogenetics interference in drug response variability, which could affect either the safety/effectiveness of COVID-19 drugs or the severity of COVID-19 progression. In this review, we will discuss in detail the pharmacogenetics of COVID-19 therapeutics with a particular focus on drugs targeting SARS-CoV-2 life cycle, drug–drug interactions (DDIs), and drug–herb interactions (DHIs) potentially affecting the pharmacogenetic interventions. We will also discuss some of the genetic variants potentially affecting the severity of COVID-19 progression.

2 VIROLOGY OF SARS-COV-2 AND ITS ENTRY INTO HUMAN CELLS

SARS-CoV-2 is a positive-sense, single-stranded RNA-enveloped virus in the *Betacoronavirus* genus (Attaway et al., 2021). Bats and pangolins may be the animal hosts of SARS-CoV-2 as there is a >90% gene homology to SARS-CoV-2 found to infect humans (Hu et al., 2021a). Currently, it remains unclear if SARS-CoV-2 was directly transferred from bat/pangolins to humans or an intermediate host was required for transmission. In light of the current pandemic, researchers

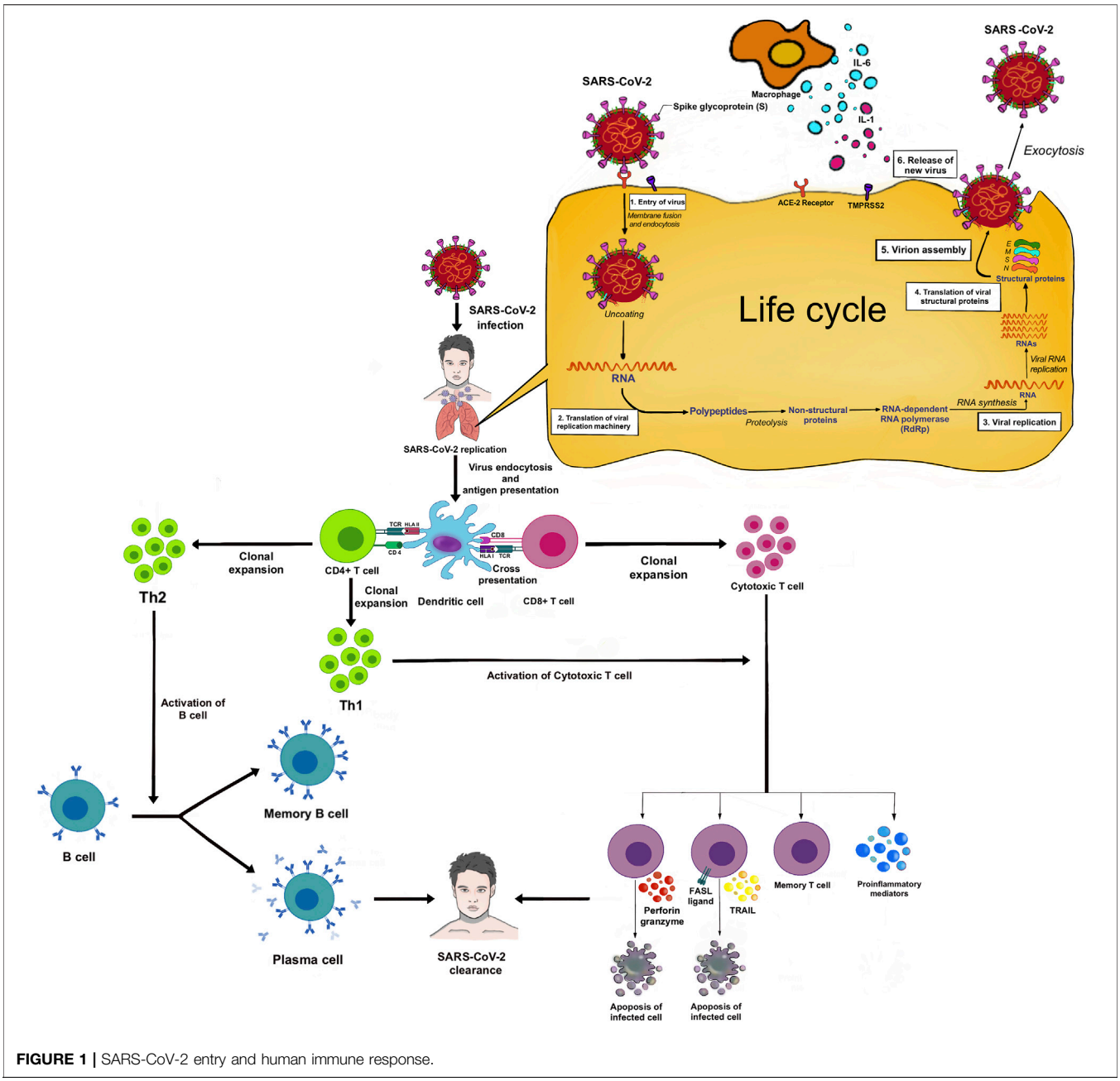

FIGURE 1 | SARS-CoV-2 entry and human immune response.

first compared SARS-CoV-2 with the previous endemic SARS-CoV (2002–2003) and MERS-CoV (2012) (da Costa et al., 2020). SARS-CoV-2 propagates and migrates down the respiratory tract along the conducting airways. The entry process of SARS-CoV-2 into host cells is *via* the binding of the S protein to the ACE2 receptor (**Figure 1**).

The virion releases its RNA. Some RNA is translated into proteins by the host cell's machinery. Proteins and RNA are assembled into a new virion in the Golgi and released. ACE2 receptors are highly expressed in the upper respiratory tract of humans (Lan et al., 2020). Proteolytic cleavage of the S protein by serine proteases including transmembrane protease serine 2 (TMPRSS2), cathepsin L, and furin is required for binding to the ACE2 receptor (Wang et al., 2020b). In the lower respiratory tract, type II pneumocytes and alveolar macrophages also express ACE2 receptors and can be infected, and release several chemokines/cytokines. Once the virus attaches to the host cell receptors, it undergoes endocytosis, viral maturation, replication, and release of more virus within the cytoplasm of the host cell. SARS-CoV-2 infection begins with viral replication and partially avoids host recognition during the initial infection and before the host innate response is enabled (Bergmann and Silverman, 2020).

Angiotensin-converting enzyme 2 (ACE2) functions as a master regulator of the renin-angiotensin system (RAS) mainly by converting Ang (angiotensin) I and Ang II into

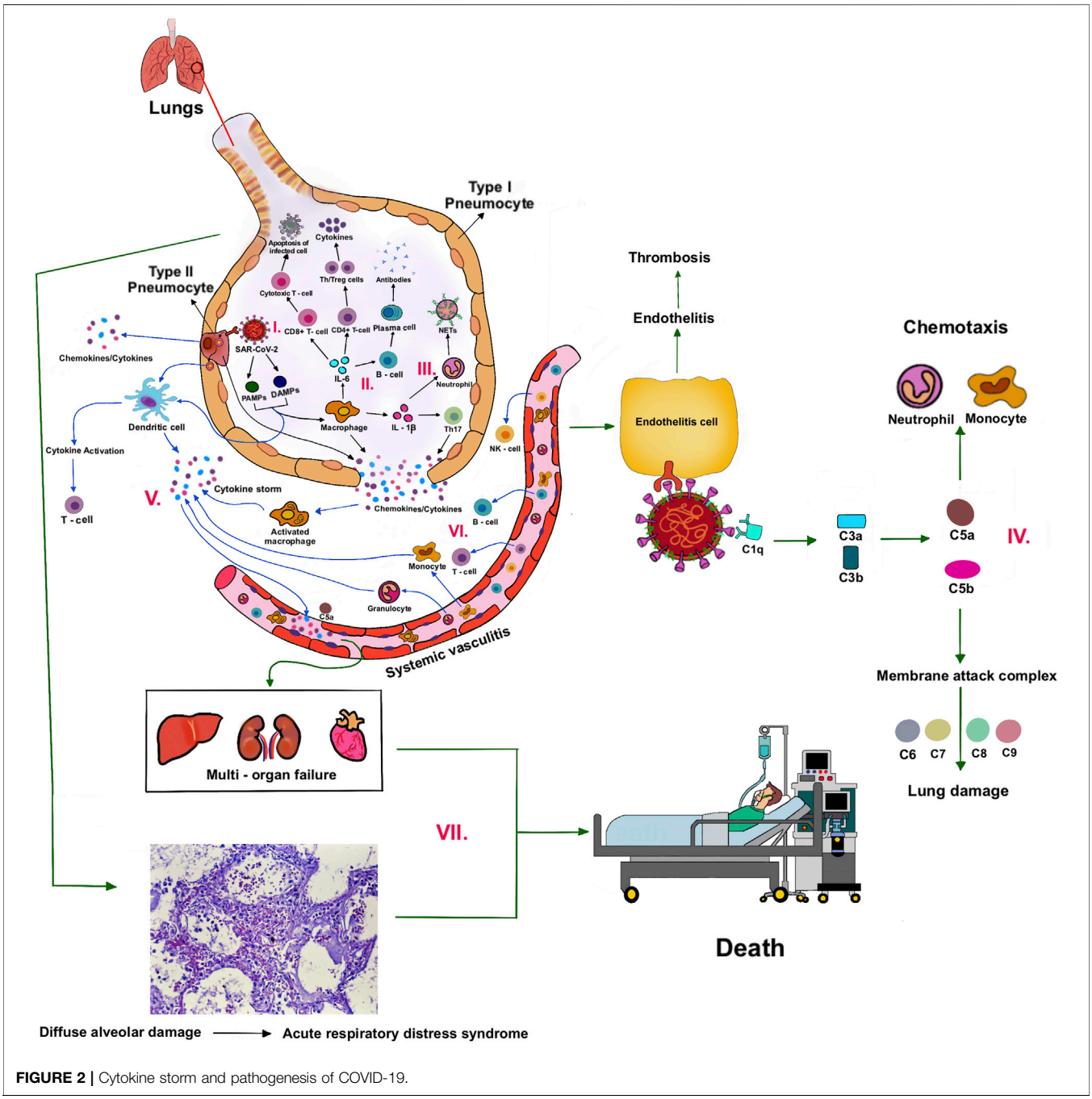

FIGURE 2 | Cytokine storm and pathogenesis of COVID-19.

Ang 1–9 and Ang 1–7, respectively. The ACE2 system is a critical protective pathway against heart failure, myocardial infarction, and hypertension, and against lung disease and diabetes mellitus. ACE2 is widely expressed, including in the lungs, cardiovascular system, gut, kidneys, central nervous system, and adipose tissue. ACE2 has recently been identified as the SARS-CoV-2 receptor (Kuhn et al., 2004). The loss of ACE2 function following binding by SARS-CoV-2 is driven by endocytosis and activation of proteolytic cleavage. Ang II levels elevate with increased activity of angiotensin 1 receptors (AT1R) at the cost of ACE2/Ang 1–7-driven pathways, leading to adverse fibrosis, hypertrophy, increased reactive oxygen species (ROS), vasoconstriction, and gut dysbiosis. ADAM17 (a disintegrin and metalloproteinase 17)-mediated proteolytic cleavage of ACE2 is upregulated by endocytosed SARS-CoV-2 spike proteins. The activation of the AT1R by elevated Ang II levels also further increases ADAM17 activity. ADAM17 correspondingly also cleaves its primary substrate releasing soluble TNF-α (tumor necrosis factor-α) into the extracellular region where it has auto- and paracrine functionality. TNF-α activation of its tumor necrosis factor receptor (TNFR)

represents a third pathway elevating ADAM17 activity (Gheblawi et al., 2020). TNF-α along with systemic cytokines released due to SARS-CoV-2 infection can lead to a cytokine storm.

3 IMMUNE RESPONSE, PATHOGENESIS, AND CLINICAL MANIFESTATION OF COVID-19

The cells of the airway epithelium are the first line of defense (innate immune system), providing a mechanical barrier (mucociliary escalator) that expels particles and pathogens *via* cilia, mucus, and induced coughing. This barrier includes cells of the pulmonary epithelium, alveolar macrophages (AMs), and dendritic cells (DCs). The AMs and DCs express pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs), that can detect molecules from pathogens (pathogen-associated molecular patterns—PAMPs) or molecules released from damaged cells (damage or danger-associated molecular patterns—DAMPs) (**Figure 2–I**).

Upon recognition, these sensors recruit the adaptor proteins, MyD88 and MAVS, respectively, and induce downstream signaling. Ultimately, this leads to the activation of the transcription factors, IRF3/7 and NF-κB, and the subsequent production of type I interferons (IFN-α and IFN-β) and pro-inflammatory cytokines (e.g., IL-6 and TNF-α), respectively. Additionally, the virus is thought to activate the inflammasome sensor, NLRP3, resulting in the secretion of the highly inflammatory cytokine IL-1β and the induction of pyroptosis, an inflammatory form of cell death (Lim et al., 2016).

T cells and B cells are activated (adaptive immune system) by antigen presentation and cytokines from DCs and AMs, and activation of the complement system. IL-6 promotes the differentiation to cytotoxic T cells, helper T cells (Th), and plasma cells. IL-1β promotes the differentiation of Th17, which functions by stimulating neutrophil recruitment and inflammation (**Figure 2–II**). Cytotoxic T cells play a crucial role in SARS-CoV-2 clearance due to their ability to selectively eliminate virus-infected cells by inducing apoptosis *via* ligands such as Fas ligand (FasL) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and perforin/granzyme-mediated pathway (Ranasinghe et al., 2016; Huang et al., 2019). Th1 helps in the activation of cytotoxic T cells. Th2 activates B cells to produce antibodies and become plasma cells. These antibodies contribute to SARS-CoV-2 clearance. There are memory T and B cells that can help against the recurrent infection (**Figure 1**).

Neutrophils are attracted by chemokines/cytokines swarm to the site of infection. Subsequently, activated neutrophils undergo degranulation and (neutrophil extracellular traps) NET formation, releasing intracellular DAMPs, DNA, histones, and neutrophil elastase that activates the PRRs of surrounding immune and non-immune cells to induce cytokine secretion (**Figure 2–III**). Neutrophils and NETs drive necro-inflammation in COVID-19 (Barnes et al., 2020). The extracellular DNA released by NETs activates platelets, and aggregated NETs provide a scaffold for binding of erythrocytes and activated platelets that promote thrombus formation (Mozzini and Girelli, 2020).

In a later phase of SARS-CoV-2 infection, the complement system will be triggered *via* antibodies bound to the virus (Noris et al., 2020). C3 can be converted into C3a and C3b. C3b mediates pathogen opsonization and activates the conversion of C5 into C5a and C5b. C5b mediates the formation of the membrane attack complex (MAC), which leads to cell lysis. C3a and C5a promote immune cell recruitment to the site of infection (**Figure 2–IV**).

Excessive cytokines produced by macrophages and DCs, that is, IL-1β, IFN-I, CXCL10, CXCL11, IL-6, IP-10, and TNF-α, are called cytokine storm (Ahmed-Hassan et al., 2020) (**Figure 2–V**). Cytokine storm and C5a lead to the influx of immune cells (e.g., granulocytes, monocytes, T cells, B cells, and NK cells) into the infected site (Wang et al., 2015) (**Figure 2–VI**). The overwhelming infiltrate of immune cells causes excessive pulmonary inflammation (severe pneumonia) with destructive effects on human tissue, resulting in destabilization of endothelial cell to cell interactions, damage of vascular barrier, capillary damage, diffuse alveolar damage (DAD), pulmonary fibrosis, systemic inflammation, hyperferritinemia, hemodynamic instability, and multi-organ failure, and if left untreated, it leads to death (Ackermann et al., 2021; Chen and Pan, 2021) (**Figure 2–VII**). Acute respiratory distress syndrome (ARDS), as a result of DAD, leads to low oxygen saturation levels and is a major cause of mortality in COVID-19. SARS-CoV-2 also can infect the endothelial cells, causing endothelial injury, endotheliitis, and microthrombus formation in several organs, especially in alveolar capillary (Varga et al., 2020). The electron microscopy shows new vessel growth through a mechanism of intussusceptive angiogenesis, especially in patients with a long duration of hospitalization (Ackermann et al., 2020). These microangiopathies could be the factors, which are worsening the ARDS. Although the exact mechanism of ARDS in COVID-19 patients is not fully understood, the excessive production of pro-inflammatory cytokines is considered to be one of the major contributing factors (Chen et al., 2020).

A common characteristic of SARS-CoV-2 is asymptomatic transmission, which is likely the cause of rampant spread and transmission. Given SARS-CoV-2 entry is primarily *via* the respiratory tract, upper and lower respiratory tract involvement is the most common manifestation. About one-third of patients hospitalized with SARS-CoV-2 infection meet the criteria for acute respiratory distress syndrome (Attaway et al., 2021). The main clinical manifestations of COVID-19 are fever (90% or more), cough (around 75%), and dyspnea (up to 50%) (Jiang et al., 2020). A small but significant subset has gastrointestinal symptoms. Preliminary estimates of case fatality, likely to fall as better early diagnostic efforts come into play, are about 2%, mostly due to ARDS, acute kidney injury, and myocardial injury (Jiang et al., 2020). The clinical manifestations are summarized in **Figure 3** as described

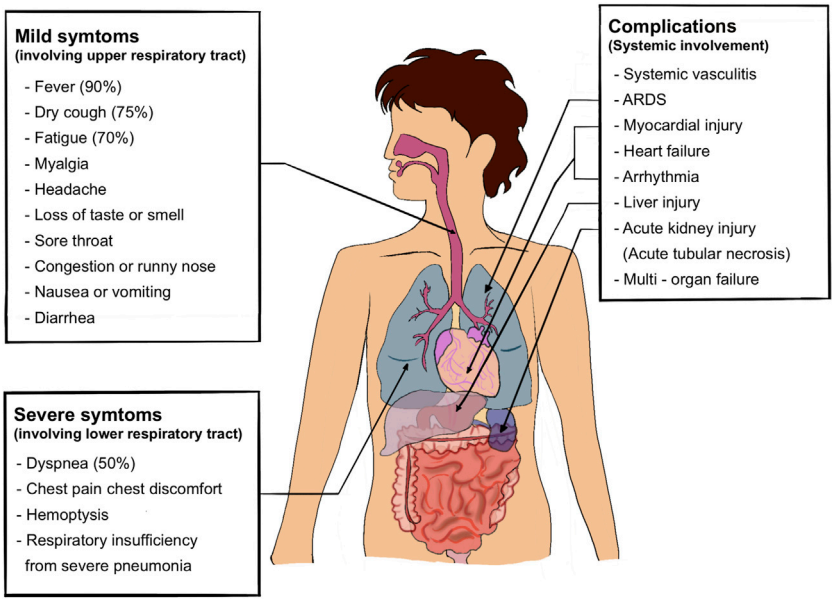

FIGURE 3 | Clinical manifestation of COVID-19.

elsewhere (Huang et al., 2020; Jiang et al., 2020; Alizadehsani et al., 2021).

4 TREATMENTS OF COVID-19

It is well recognized that COVID-19 has four stages of progression in which the first stage is initiated by upper respiratory tract infection. In the second stage, the symptoms of dyspnea and pneumonia appeared. In the third stage of COVID-19, cytokine storm followed by the hyperinflammatory state predominantly worsens the clinical scenario. The final stage of COVID-19 progression is either death or recovery. While as many as 800 clinical trials are ongoing and some of these have already been completed worldwide, currently, no treatment was found to be clinically effective to act selectively against the SARS-CoV-2 infection (Becker, 2020; Stasi et al., 2020). Currently, different therapeutics are being applied to treat moderate-to-severe COVID-19 patients considering the pathological features and various stages of COVID-19, of which repurposed drugs are being used predominantly (Becker, 2020; Song et al., 2020; Stasi et al., 2020; Gavriatopoulou et al., 2021).

5 REPURPOSING DRUGS FOR COVID-19: CONCEPT AND MECHANISM OF ACTION

Gilead Sciences first developed remdesivir (RDV) in 2017 for the treatment of infection caused by Ebola virus. In the United States, South Korea, and China, RDV was clinically evaluated in moderate-to-severe COVID-19 patients through several phase 3 clinical trials (Gavriatopoulou et al., 2021). Based on reviewing current evidence from randomized, double-blinded, placebo-controlled clinical trials, the FDA has been persuaded to believe the potential benefits of RDV over potential risks for the treatment of severe hospitalized COVID-19 patients (FDA, 2020). Henceforth, the FDA issued an Emergency Use Authorization (EUA) for emergency use of RDV for the treatment of hospitalized severe COVID-19 adult and children patients where severity of COVID-19 has been defined as $SpO_2 \leq$ 94% on room air, requiring supplemental oxygen, mechanical ventilation, or extracorporeal membrane oxygenation (FDA, 2020). It is reported that RDV can inhibit RNA-dependent polymerase and may therefore be effective in the treatment of SARS-CoV-2 infection. It is actually a phosphoramidate prodrug having broad-spectrum activity against various viruses, for example, Paramyxoviridae, Filoviridae, Pneumoviridae, and Orthocoronavirinae, that is, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV), as described elsewhere (Sheahan et al., 2017; Martinez, 2020; Gavriatopoulou et al., 2021). Although the FDA has recommended emergency use of RDV in severe COVID-19 patients, however, the safety and efficacy of RDV in COVID-19 patients as evidenced in multiple recent meta-analyses are controversial and inconsistent (Angamo et al., 2021; Elsawah et al., 2021; Tao et al., 2021).

Chloroquine (CQ) and hydroxychloroquine (HCQ) were included on the essential lists of medications of the World Health Organization (WHO) and used for several decades for the prophylaxis of malaria. They are also used for the treatment of rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, and post-Lyme's disease arthritis (Shippey et al., 2018; Schrezenmeier and Dörner, 2020; Gavriatopoulou et al., 2021). Although HCQ and CQ may exhibit anti-inflammatory, immunomodulating, anti-infective, antithrombotic, and

metabolic effects, however, they can also inhibit SARS-CoV-2 host entry by binding to the host cell angiotensin-converting enzyme-2 (ACE2) receptor, thereby impairing SARS-CoV-2 spike protein recognition (Becker, 2020; Fantini et al., 2020). These drugs act by blocking 2019-nCoV entry into host cells by inhibiting glycosylation of host receptors, proteolytic processing, and endosomal acidification (Sanders et al., 2020). Several clinical trials had assessed the safety and efficacy of CQ/HCQ in COVID-19 patients. Based on primary results from some clinical trials, the U.S. FDA had approved emergency use of CQ/HCQ for the treatment of COVID-19 patients on 28 March 2020. Later, the U.S. FDA issued a cautioning statement against CQ/HCQ use in COVID-19 patients due to serious cardiac toxic effects, for example, arrhythmias on April 24, 2020. Finally, on 15 June 2020, the FDA revoked the EUA use of CQ/HCQ as a potential COVID-19 therapy after accumulating negative data from clinical trials (Gavriatopoulou et al., 2021).

Lopinavir (LPV) and ritonavir (RTV) are HIV protease inhibitors and used in combination with or without other antiviral drugs for the treatment of human immunodeficiency virus (HIV-1)-infected patients older than 2 years. Due to its inhibiting nature of viral DNA-dependent RNA polymerase, either combination of LPV/RTV or alone has been recommended for the treatment of COVID-19 patients; however, the results of clinical trials are not favoring the clinical outcomes and are limited in use nowadays (Cao et al., 2020; Gavriatopoulou et al., 2021). Although LPV/RTV was suggested primarily by the National Health Commission (NHC) of China as an antiviral therapy in COVID-19 patients, however, it is not recommended by the U.S. National Institute of Health (NIH) due to lack of proven clinical effectiveness in these patients (Song et al., 2020).

Arbidol was first marketed in Russia and China as a synthetic antiviral drug for the treatment of seasonal influenza. A previous study demonstrated that arbidol was broadly effective against some other viruses including SARS-CoV and was generally well tolerated in treating these viruses (Gavriatopoulou et al., 2021). Initially, the *in vitro* test found arbidol to be an effective inhibitor of SARS-CoV-2 infection, and it was therefore recommended by the China's NHC guide on COVID-19 treatment option. It appeared that arbidol was found ineffective against SARS-CoV-2 infection in ongoing clinical studies, although it had significant limitations in study design and sample size in these studies (Song et al., 2020).

Favipiravir was first approved in Japan for the treatment of influenza and was later found effective against Ebola virus infection also. Although several clinical trials were undertaken in China, Japan, Canada, and Russia evaluating the safety and efficacy of favipiravir alone or in combination with other antivirals against SARS-CoV-2 infection, however, the results were not persuading the clinicians for further considerations in treating COVID-19 patients with favipiravir (Gavriatopoulou et al., 2021).

Originally, darunavir/cobicistat was developed for the treatment of HIV-1 infection. Due to its protease inhibiting activity, the clinical trial had assessed the safety and efficacy of darunavir/cobicistat in SARS-CoV-2 infection and found that darunavir/cobicistat was not effective in the treatment of COVID-19 patients (Gavriatopoulou et al., 2021).

It is worth mentioning here that there are no supporting data from clinical trials that could favor the use of any HIV protease inhibitors to treat COVID-19 patients. Followingly, recently, the NIH panel for COVID-19 treatment guidelines did not recommend the use of any HIV protease inhibitors in the treatment of COVID-19 infection due to lack of clear clinical benefit in these patients (Amanat and Krammer, 2020; Gavriatopoulou et al., 2021).

Atazanavir (ATV) was discovered early in the 2000s as an antiretroviral drug for treating HIV instead of LPV/RTV because of lesser side effects of this drug. Evidence from *in silico* and *in vitro* studies suggests that by inhibiting viral major protease, ATV would inhibit SARS-CoV-2 replication even better than LPV/RTV (Fintelman-Rodrigues et al., 2020; Stasi et al., 2020; Alavian et al., 2021).

As part of highly active antiretroviral therapy (HAART), efavirenz (EVZ) and nevirapine are mainly used in the treatment of HIV/AIDS; however, these drugs could also be used for treating SARS-CoV-2 infection because of their ability to inhibit viral RNA-dependent RNA polymerase (RdRp) (Nastri, et al., 2020). Also, nelfinavir mesylate (NFV) being an antiretroviral drug may have potential efficacy against SARS-CoV-2 infection. Recent studies suggest that it can inhibit spike protein (S) medicated cell fusion of SARS-CoV-2 and may eventually block the transfer and cell-to-cell spread of SARS-CoV-2 (Yousefi et al., 2021).

Ribavirin is a nucleoside analog and was found effective against many RNA viruses, including SARS-CoV and MERS-CoV. It mainly inhibits RNA polymerase and synthesis of viral protein. Ribavirin was widely used with or without steroids against SARS infection, outbreak in 2003. Although intravenous ribavirin in combination with LPV/RTV or interferon was suggested by China's NHC for the treatment of patients with COVID-19, it is not recommended by the NIH (Song et al., 2020).

It has well established that severe COVID-19 patients are generally associated with an increased cytokine-release syndrome, which further elevated interleukin-6 (IL-6). Tocilizumab, an IL-6 receptor antagonist, is commonly used for the treatment of rheumatoid arthritis and patients having cytokine-release syndrome (Takahashi et al., 2020). It was authorized by the Agenzia Italiana del Farmaco (AIFA), the Italian Medicines Agency, to investigate its safety and efficacy in COVID-19 patients. However, a recent clinical trial did not find significant clinical benefits, for example, reduced mortality or increased survival rate in using this drug (Stasi et al., 2020; Salama et al., 2021).

Molnupiravir, a ribonucleoside prodrug of N-hydroxycytidine (NHC), was originally developed as a potent inhibitor of respiratory syncytial virus (RSV), influenza B viruses, and influenza A viruses (IAVs) of human, avian, and swine origins (Yoon et al., 2018; Jayk Bernal et al., 2021). Later, it was found to be effective as a first oral and direct-acting anti-SARS-CoV-2 agent in both *in vitro* and *in vivo* studies (Jayk Bernal et al., 2021; Kabinger et al., 2021). When molnupiravir in the form of NHC prodrug is administered orally, it circulates systemically and is phosphorylated intracellularly to NHC triphosphate, an active

TABLE 1 | Possible mechanism of actions of COVID-19 therapeutics.

COVID-19 therapeutics	Potential mechanism of action against SARS-CoV-2 infection	Reference
Camostat mesilate	Protects viral entry by inhibiting TMPRSS2	Gunst et al. (2021)
Arbidol/umifenovir	Inhibits membrane fusion of the viral envelope	Yousefi et al. (2021)
Lopinavir/ritonavir/darunavir and atazanavir	Viral protease inhibitors	Alavian et al. (2021)
Remdesivir	It binds to the viral RNA-dependent RNA polymerase (RdRp), inhibiting viral replication through premature RNA transcription termination	Fricke-Galindo and Falfán-Valencia, (2021)
Favipiravir	It inhibits RdRp and synthesis of viral protein	Sanders et al. (2020)
Ribavirin	It inhibits RdRp and synthesis of viral protein	Sanders et al. (2020)
Efavirenz	Inhibits RdRp	Nastri et al. (2020)
Nevirapine	Inhibits RdRp	Nastri et al. (2020)
Tocilizumab	Reduces cytokine release by inhibiting IL-6 receptor	Takahashi et al. (2020)
Sarilumab and siltuximab	IL-6 antagonist	Yousefi et al. (2021)
Anakinra	IL-1 antagonist	Yousefi et al. (2021)
Ruxolitinib and baricitinib	Acts as an immunomodulator by inhibiting of Janus kinases and may therefore reduce the transduction of the cytokine-mediated signal	Stasi et al. (2020), Takahashi et al., (2020)
Ivermectin	Inhibits viral replication	Yousefi et al. (2021)
Oseltamivir	By inhibiting neuraminidase distributed on the surface of the virus, block viral release	Badary (2021), Yousefi et al. (2021)
Molnupiravir	Through RNA mutagenesis	Gordon et al. (2021), Kabinger et al. (2021)

form of molnupravir. This active form subsequently interferes in viral replication by inducing RNA mutagenesis through incorporation of 5′-monophosphate metabolite into viral RdRp. The active compound of molnupiravir, NHC 5′-triphosphate (NHC-TP), increases "G" to "A" and "C" to "U" transition mutations in replicating coronaviruses that lead to increased antiviral effects (Ehteshami et al., 2017; Gordon et al., 2021; Jayk Bernal et al., 2021; Kabinger et al., 2021).

Another important treatment strategy against SARS-CoV-2 infection was to develop selective targets that may neutralize monoclonal antibodies (mAbs) since previous studies reported a large number of antibodies by disturbing the receptors of either SARS-CoV or MERS coronavirus (MERS-CoV) that showed neutralization activities (Du et al., 2009; de Wit et al., 2016). Generation of virus-neutralizing antibodies or neutralizing mAbs (from the B cells of convalescent patients or humanized mice sources) are being developed from B cells of convalescent patients or humanized mice against viral infections, by targeting the receptor-binding domain (RBD) of the spike (S) protein of SARS-CoV-2 by some mechanisms—directly through triggering the phagocytosis by binding to virons or infected cells—and also by two different types of distance mechanisms in an antibody-dependent enhancement (ADE) process:

a) ADE *via* enhanced infection-expanded viral disease and replication by viral uptake into Fc gamma receptor IIa (FcγRIIa)-expressing phagocytic cells.
b) ADE *via* enhanced immune activation by excessive antibody Fc-mediated effector functions or immune complex formation in an antibody-dependent manner (Lee et al., 2020; Taylor et al., 2021).

To date, seven mAb neutralizing drugs including bamlanivimab, etesevimab, casirivimab, imdevimab, sotrovimab, cilgavimab, and tixagevimab either as monotherapy or as combination therapy have been approved or received EUAs from the U.S. FDA for the treatment of COVID-19 (Agarwal et al., 2020; Shi et al., 2020a; Zhang et al., 2021c; Nathan et al., 2021). All repurposed drugs with their mechanism of actions against SARS-CoV-2 infections are shown in **Table 1**.

Also, the different drugs acting on different phases of SARS-CoV-2 life cycle are shown in **Figure 4**

6 POTENTIAL RISK OF COVID-19 AND SUPPORTIVE TREATMENTS

6.1 Anticoagulants

One of the most emerging prevalent risks associated with SARS-CoV-2 severe infection is venous thromboembolism (VTE), particularly pulmonary embolism (PE). The reported prevalence of VTE is ~25–30% in severe COVID-19 patients, which is considerably higher than that of other viral infections (Bikdeli et al., 2020; Klok et al., 2020; Gavriatopoulou et al., 2021). Regulatory-approved drugs such as direct oral anticoagulants (DOACs), for example, rivaroxaban, apixaban, and dabigatran, and vitamin K antagonists, for example, warfarin, could be used to minimize the risk of VTE in severe COVID-19 patients. These supportive therapies should be continued for at least 3 months if VTE is suspected or confirmed in COVID-19 patients (Bikdeli et al., 2020; Gavriatopoulou et al., 2021).

6.2 Angiotensin-Converting Enzyme Inhibitors/Angiotensin Receptor Blockers

Initially, there was a great concern whether angiotensin-converting enzyme inhibitors (ACEIs)/angiotensin receptor blockers (ARBs) should or should not be continued in COVID-19 patients, especially with hypertension and diabetes mellitus. This is because SARS-CoV-2 binds to the ACE2 receptor to gain entry into the host cells. However, recent meta-analyses established that COVID-19 patients taking ACEIs/ARBs were not associated with an increased risk of mortality compared to those

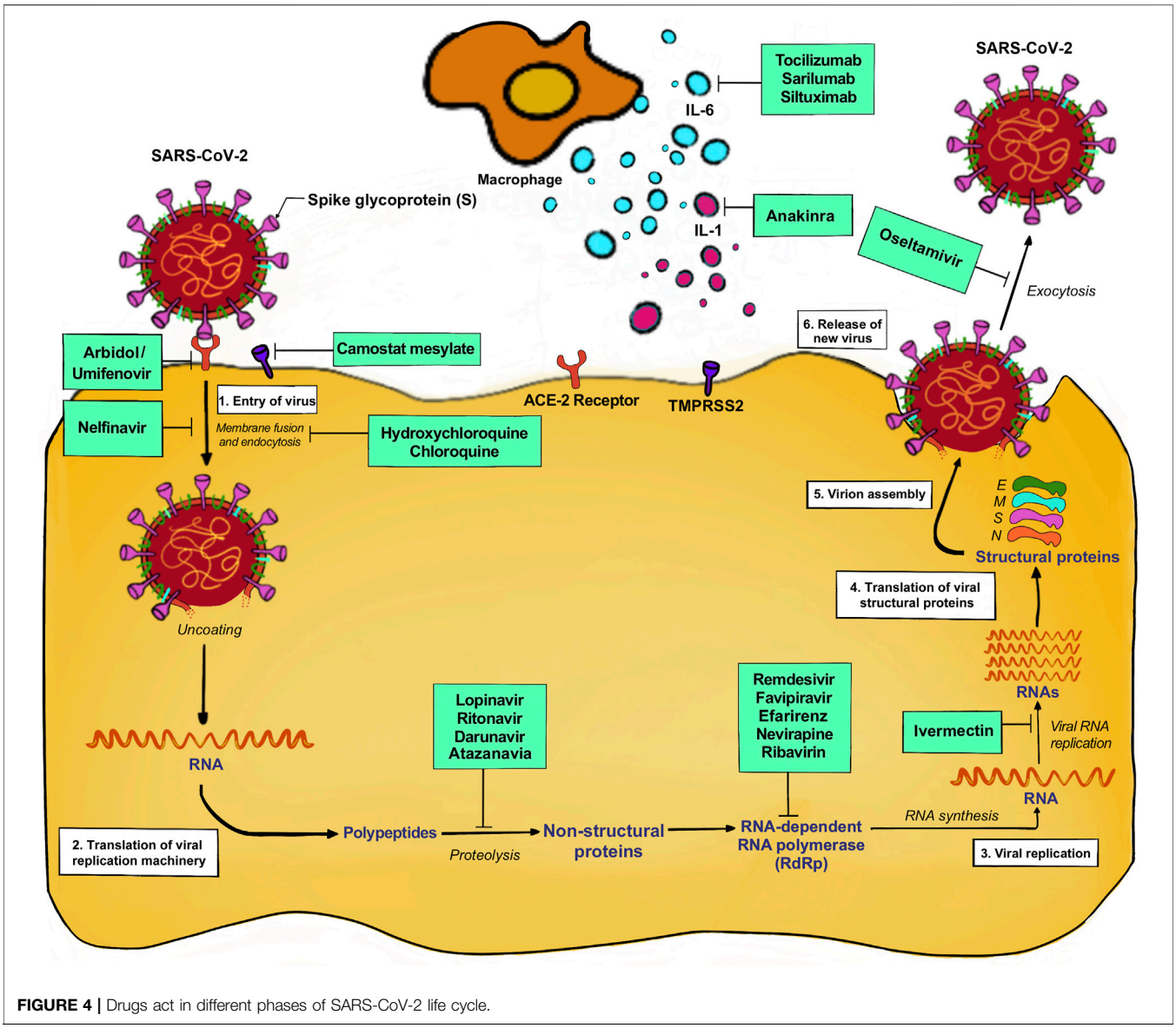

FIGURE 4 | Drugs act in different phases of SARS-CoV-2 life cycle.

not taking ACEIs/ARBs. The risk of composite severe clinical manifestations was not significantly different between the positive patients with or without ACEI/ARB users and also found evidence of beneficial effects for using ACEIs/ARBs especially in hypertensive COVID-19 patients. These results strongly suggest continuing with renin angiotensin aldosterone system (RAAS) inhibitors during the COVID-19 pandemic (Baral et al., 2020; Biswas and Kali, 2021a; Wang et al., 2021).

6.3 Antiplatelets

A recent meta-analysis indicated that the risk of acute stroke was significantly higher in severe COVID-19 patients than in non-severe COVID-19 patients (RR = 4.18, 95% CI: 1.7–10.25; p = 0.002) (Siepmann et al., 2021). Clinical studies also showed that heart failure/myocardial infarction (MI) is prevalently higher in severe COVID-19 patients. The P2Y12 receptor antagonists, for example, clopidogrel, prasugrel, and ticagrelor, are widely used as first-line therapy in patients with stroke or coronary artery disease (CAD) (Bikdeli et al., 2020; Sivaloganathan et al., 2020; Zhao et al., 2021).

6.4 Antifibrotics

Idiopathic pulmonary fibrosis is one of the major risk factors associated with the severity of COVID-19. Magnitude and intensity of lung fibrosis may increase the risk for severe clinical outcomes in patients with COVID-19. It is proposed that antifibrotics such as pirfenidone and nintedanib may reduce the severity of SARS-CoV-2 infection and might be an integral part of COVID-19 therapeutics (Bikdeli et al., 2020; Gavriatopoulou et al., 2021).

6.5 Systemic Corticosteroids

During severe acute respiratory syndrome coronavirus (SARS-CoV) outbreak in 2002–2004, steroid therapy was commonly

administered along with other medications. Initially, the WHO did not support their use without the results of clinical trials being assessed and only recommended their strict use in especial clinical circumstances. However, with the progression of the pandemic, robust evidence for the associations of corticosteroids with the clinical outcomes in COVID-19 is becoming available since steroids are being currently administered in many parts of the world (Mattos-Silva et al., 2020).

A recent retrospective study indicated that methylprednisolone was associated with a decreased risk of death (HR 0.38; 95% CI 0.20–0.72) in patients with severe COVID-19, who developed ARDS (Wu et al., 2020). Another retrospective study revealed that COVID-19-hospitalized patients taking steroids were associated with a significantly lower mortality rate than those who did not take steroids (13.9 vs. 23.9%; HR 0.51, 95% CI 0.27–0.96, p = 0.044) (Fernández-Cruz et al., 2020). Very recently, an open-label randomized controlled trial (RCT) showed that COVID-19-hospitalized patients taking dexamethasone were associated with a significantly lower rates of 28-day mortality than the patients taking standard of care (RR 0.83, 95% CI 0.74–0.92, p = 0.0007). This study further revealed that dexamethasone reduced mortality significantly in ventilated COVID-19 patients (RR 0.65, 95% CI 0.48–0.88, p = 0.0003) as well as in patients who have taken supplemental oxygen (RR 0.80, 95% CI 0.67–0.96, p = 0.0021) (Horby et al., 2021).

Systemic steroids especially dexamethasone in specific COVID-19 patients, for example, critically ill or require supplemental oxygen, may be considered based on the current available evidence. The clinical benefits of dexamethasone use may be apparent in COVID-19 patients if they were treated for greater than 7 days after the onset of COVID-19-related symptoms (Gavriatopoulou et al., 2021).

6.6 Bronchodilators/Vasodilators

Bronchodilators may be administered whenever indicated in COVID-19 patients. Severe COVID-19 patients with hypoxemia may be particularly benefited from the pulmonary vasodilators. Although the lack of rigorous evidence did not favor the use of pulmonary vasodilators, for example, nitric oxide in COVID-19 patients, a recent, open-label, parallel-group, phase 2, RCT indicated that early inhalation of budesonide reduced the risk of urgent medical care support and also reduced the time to recover from early COVID-19 diagnosis (Gavriatopoulou et al., 2021; Ramakrishnan et al., 2021).

6.7 Non-Steroidal Anti-Inflammatory Drugs

Since fever and pain are common in SARS-CoV-2 infection, paracetamol should be generally considered as a first-line antipyretic and analgesic agent if not contraindicated due to other clinical conditions. However, ibuprofen may be reserved for patients who are unable to tolerate paracetamol until further studies clarify the adverse and beneficial effects of non-steroidal anti-inflammatory drugs (NSAIDs) in patients with COVID-19 (Robb et al., 2020; Gavriatopoulou et al., 2021).

7 PHARMACOGENOMICS AND PRECISION MEDICINE FOR COVID-19

Pharmacogenomic considerations of currently used COVID-19 therapeutics may help clinicians to optimize the efficacy or safety of these drugs, and may accelerate the development of precision COVID-19 medicine. To mitigate devastating catastrophe associated with COVID-19, many drugs without establishing robust evidence of efficacy or magnitude of toxicities have been used in these patients either as an off-label/compassionate use or as a clinical trial under these unprecedented health situations as an urgent attempt. Although pharmacogenomic determinants are very important considerations in optimizing the efficacy or toxicity of many repurposed antiviral drugs or supportive treatments, they are the most neglected issues in COVID-19 therapeutics since there are almost no pharmacogenomics data for these drugs (Takahashi et al., 2020). It is very unlikely that pharmacogenomic associations of COVID-19 therapeutics with the efficacy or safety have not been considered yet in any clinical studies.

8 PHARMACOGENOMIC LANDSCAPE OF COVID-19 THERAPIES

8.1 Genetic Variants Affecting the Safety or Efficacy of COVID-19 Therapies

8.1.1 Drug-Metabolizing Enzymes

Many drugs either repurposed antivirals or supporting medications that are used in the treatment of COVID-19 are metabolized by a number of drug-metabolizing enzymes called cytochrome P450 (CYP) enzymes. Genetic variants of the *CYP* genes encoding these important CYP enzymes may regulate their expression and may also contribute to drug response variability by altering the PK properties of the respective drugs. Therefore, for achieving optimal efficacy or safety of COVID-19 therapeutics, *CYP* genes of interest should be considered in future clinical studies to investigate such genetic associations. Here, we will summarize all *CYP* genes with potential interest of single-nucleotide polymorphisms (SNPs) as well as other genes affecting the PK profile of COVID-19 therapeutics with potential considerations of pharmacogenomics (PGx) of these drugs in other clinical conditions as evidenced from the literature (**Table 2**).

It is important to recognize that *CYP* genetic variants are highly polymorphic and only few of these genetic variants are associated with the safety or efficacy of the respective drugs. The most prevalent and studied genetic variants of the *CYP3A4/5*, *CYP2B6*, *CYP2C8*, *CYP2C9*, *CYP2C19*, and *CYP2D6* pharmacogenes have wide inter-ethnic variability. For example, *CYP2D6*4* is highly prevalent in American population, whereas *CYP2C19*2*, **3* is highly prevalent in South Asians (Sukasem et al., 2013; Medhasi et al., 2016; Zhou et al., 2017; Biswas, 2021a; Sukprasong et al., 2021).

8.1.2 Transporters

In addition to *CYP* genes, some other efflux or uptake transporter proteins encoded by the transporter genes may also modify the

TABLE 2 | Evidence of pharmacogenetic associations of COVID-19 therapies in other disease conditions.

Drug	Gene	SNP	Effects on PK/safety/efficacy	MAF using 1,000 genome database	Disease	Reference
Atazanavir	*SLCO1B1*	rs4149056	Enhanced toxicity	C = 8.8%	HIV	Bonora et al. (2015)
	ABCB1	rs2032582	Hyperbilirubinemia	A = 33.4%	HIV	Rodríguez-Nóvoa et al. (2007)
	UGT1A1	rs8175347	Hyperbilirubinemia	AT = 34.8%	HIV	Rodríguez-Nóvoa et al. (2007), Du et al. (2019)
	CYP3A5	rs2740574	Efficacy	C = 23.1%	HIV	Cafiero et al. (2020)
Azithromycin	*ABCB1*	rs1045642	Variability in AUC	A = 39.5%	Healthy volunteers	He et al. (2009)
Clopidogrel	*CYP2C19*	rs4244285	Efficacy	A = 22.1%	ACS	Scott et al. (2013)
Dexamethasone	*UGT1A1*	rs4148323	Efficacy	A = 3.4%	Cancer	Yamasaki et al. (2015)
	ABCB1	rs1045642	Efficacy	A = 39.5%	Autoimmune disease	Song et al. (2017)
Darunavir	*SLCO3A1*	rs8027174	PK	T = 4.7%	HIV	Moltó et al. (2013)
Efavirenz	*CYP2B6*	rs3745274	CNS toxicity, suicide attempt	T = 31.6%	HIV	Sukasem et al. (2012), McDonagh et al. (2015), Desta et al. (2019)
	ABCB1	rs3842	PK	C = 18.8%	Healthy volunteers	Mukonzo et al. (2009)
Ivermectin	*CYP3A4*	rs35599367	PK	A = 1.5%	Healthy volunteers	González Canga et al. (2008)
	ABCB1	rs1045642	PK	A = 39.5%	A549 cell lines	Lespine et al. (2006)
Lopinavir	*SLCO1B3*	rs717620	Dyslipidemia and hyperbilirubinemia	T = 13.5%	HIV	da Rocha et al. (2015)
	ABCC2	rs8187710	Dyslipidemia and hyperbilirubinemia	A = 6.8%	HIV	Lubomirov et al. (2010)
	SLCO1B1	rs4149056	Efficacy	C = 8.8%	HIV	Lubomirov et al. (2010)
	ABCB1	rs1045642	Safety/efficacy	A = 39.5%	HIV	Rakhmanina et al. (2011)
	CYP3A5	rs2740574	Efficacy	C = 23.1%	HIV	Rakhmanina et al. (2011)
Losartan	*CYP2C9*	*rs1799853*	PK	T = 4.8%	Cell lines	Iwamura et al. (2011)
	ABCB1	rs1045642	Efficacy	A = 39.5%	Hypertension	Göktaş et al. (2016)
Ribavirin	*SLC28A2*	rs11854484	Anemia	T = 30.3%	HCV	Allegra et al. (2015)
Ritonavir	*ABCB1*	rs1045642	PK	A = 39.5%	HIV	Rakhmanina et al. (2011)
	CYP3A5	rs2740574	Efficacy	C = 23.1%	HIV	Rakhmanina et al. (2011)
	SLCO1B1	rs4149056	Efficacy	C = 8.8%	HIV	Lubomirov et al. (2010)
Umifenovir	*CYP3A4*	rs35599367	PK	A = 1.5%	Healthy volunteers	Deng et al. (2013)
Warfarin	*CYP2C9*	rs1799853	Toxicity	T = 4.8%	Thromboembolism	Johnson et al. (2017)

Here, SNP, single-nucleotide polymorphism; PK, pharmacokinetic; MAF, Minor allele frequency; AUC, area under concentration; SLE, systemic lupus erythematosus; DLE, discoid lupus erythematosus; ACS, acute coronary syndrome.

PK properties of COVID-19 therapeutics and may be associated with the efficacy or safety variability. Like *CYP* genetic variants, transporter genes such as *ABCB1*, *SLCOIB1*, and *ABCC2* also have interindividual variabilities and may affect the safety or efficacy of drugs accordingly (Sensorn et al., 2013; Medhasi et al., 2016; Atasilp et al., 2020; Biswas, 2021b). The list of transporter genes with relevant COVID-19 drugs with potential considerations of PGx in other clinical conditions as evidenced from the literature is shown in **Table 2**.

8.2 Other Genes Affecting the Severity of SARS-CoV-2 Infection

8.2.1 *HLA*

The human leukocyte antigen (HLA) encoded by the *HLA* gene is located on chromosome 6p21 which contains crucial regulators of immune response. The classical genes *HLA-A*, *HLA-B*, and *HLA-C* are in Class I and the classical genes *HLA-DP*, *HLA-DQ*, and *HLA-DR* are in Class II (Wang et al., 2015). HLA Class I has a role to present pathogen peptides to $CD8^+$ T cell, becoming cytotoxic T cell which can directly destroy infected cell by inducing apoptosis (cellular immunity), whereas HLA class II has a role to present pathogen peptides to $CD4^+$ T cell, activating B cell to become plasma cell and produce antibodies (humoral immunity) (**Figure 1**). However, in the case of viral infection such as COVID-19, cellular immunity is more important than humoral immunity to clear out the viruses which are staying inside the host cells (Le Bert et al., 2020).

Some variation of *HLA* alleles also has an association with some certain disease. The recently introduced genome-wide association study (GWAS) has suggested that several genes converging in common pathways contribute to the genetic susceptibility in several disorders, such as ankylosing spondylitis, psoriasis, chronic beryllium disease, rheumatoid arthritis, celiac disease, type 1 diabetes, and multiple sclerosis (Fiorillo et al., 2017). For example, 90–95% of patients with ankylosing spondylitis have *HLA-B27* (Zhu et al., 2019). *HLA* alleles are not only associated with a set of autoimmune disease; the study in Thai children with autism showed that *HLA-B*13:02*, *HLA-B*38:02*, *HLA-B*44:03*, and *HLA-B*56:01* alleles were significantly increased in autistic subjects compared with normal subjects (Puangpetch et al., 2015).

In addition to an association with some diseases, *HLA* alleles are also associated with an increased risk of certain drug allergy (Sukasem et al., 2014b; 2018b). The study in Thai population shows that *HLA-B*15:02* is strongly associated with aromatic antiepileptic drug (AED)-induced Stevens-Johnson syndrome

TABLE 3 | Summary of the pharmacogenomic studies affecting the safety or efficacy of COVID-19 therapeutics in other clinical conditions.

COVID-19 therapeutics	Potential genes of interest affecting PK/PD properties	Already assessed gene/genetic variants	Effects on safety or efficacy	Disease condition	Reference
Atazanavir	*UGT1A1* and *ABCB1* *APOA5*, *APOC3*, *ABCA1*, and *APOE*	*UGT1A1* and *ABCB1* *APOA5*, *APOC3*, *ABCA1*, and *APOE*	Hyperbilirubinemia and dyslipidemia	HIV	(Gammal et al., 2016; Badary, 2021)
Efavirenz	*CYP2B6* and *ABCB1*	*CYP2B6*	Depression and suicidal tendencies	HIV	(Sukasem et al., 2012; Desta et al., 2019)
Oseltamivir	*ABCB1*, *CES1*, *NEU2*, and *SLC15A1*	*CES1* and *ABCB1*	AUC and toxicity	ARDS	(Tarkiainen et al., 2012; Bermúdez de León et al., 2020)
Ivermectin	*ABCB1*, *SLCO1A2*, and *SLCO2B1*	*ABCB1*	Neurologic Toxicity	Patients with parasite infection	(Baudou et al., 2020; Fricke-Galindo and Falfán-Valencia, 2021)
Tocilizumab	FCGR3A, IL6R, CD69, and GALNT18	FCGR3A	Higher response	RA	Takahashi et al. (2020)
Nevirapine	*HLA*, *CYP2B6*, and *ABCB1*	*HLA*, *CYP2B6*, and *ABCB1*	SJS/TEN and hepatotoxicity	HIV	(Martin et al., 2005; Vitezica et al., 2008)
Interferon (INF) β-1b	*IRF6*	*IRF6*	Liver injury	multiple sclerosis	Kowalec et al. (2018)
Azithromycin	*ABCB1*	*ABCB1*	~2-fold lower peak conc	Healthy volunteers	He et al. (2009)
Clopidogrel	*CYP2C19* *ABCB1*	*CYP2C19* and *ABCB1*	MACE	CAD	(Sukasem et al., 2013; Biswas et al., 2020a; Biswas and Kali, 2021b)
Warfarin	*CYP2C9* and *VKORC1*	*CYP2C9* and *VKORC1*	Efficacy and toxicity	Thrombotic patients	Johnson et al. (2017)
Apixaban, dabigatran, and rivaroxaban	*ABCB1*	*ABCB1*	Efficacy and toxicity	Thrombotic patients	(Xie et al., 2018; Kanuri and Kreutz, 2019)
Losartan	*CYP2C9* *ABCB1*	*CYP2C9* and *ABCB1*	Toxicity Efficacy	Hypertension	(Iwamura et al., 2011; Göktaş et al., 2016)
NSAIDs	*CYP2C9*	*CYP2C9*	Toxicity	Patients with pain	Theken et al. (2020)

Here, NSAIDs, non-steroidal anti-inflammatory drugs; CAD, coronary artery disease; RA, rheumatoid arthritis; MACE, major adverse cardiovascular events; ARDS, acute respiratory distress syndrome.

(SJS)/toxic epidermal necrolysis (TEN) (Sukasem et al., 2021b); *HLA-B*15:02* and *HLA-C*08:01* alleles are significantly associated with co-trimoxazole (CTX)-induced SJS/TEN, whereas the *HLA-B*13:01* allele was significantly associated with CTX-induced drug reaction with eosinophilia and systemic symptoms (DRESS) (Sukasem et al., 2020a); *HLA-B*46:01*, *HLA-B*56:02/04*, and *HLA-B*40:01* alleles contribute to the risk of phenytoin-induced cutaneous adverse drug reactions (PHT-induced cADRs) (Sukasem et al., 2020b); carbamazepine-induced SJS/TEN shows an association with *HLA-B*15:21* allele (Jaruthamsophon et al., 2017; Sukasem et al., 2018a); *HLA-A*02:07* and *HLA-B*15:02* are associated with lamotrigine (LTG)-induced cutaneous adverse drug reactions (cADRs) (Koomdee et al., 2017); *HLA-B*13:01* is associated with dapsone-induced severe cutaneous adverse reactions including SJS/TEN and DRESS (Tempark et al., 2017); and *HLA-B*58:01* is associated with allopurinol-induced SJS/TEN, and screening for *HLA-B*58:01* alleles in patients who will be treated with allopurinol would be clinically helpful in preventing the risk of developing cADRs (Sukasem et al., 2016b). The future of pharmacogenomics-guided therapy in clinical settings across Thailand appears promising because of the availability of evidence of clinical validity of the pharmacogenomics testing (Sukasem et al., 2021a). The effectiveness of *HLA* screening on a wider scale in clinical practice requires significant resources, including state-of-the-art laboratory; multidisciplinary team approach, and healthcare provider education and engagement; clinical decision support alert system *via* electronic medical record (EMR); laboratory standards and quality assurance; evidence of cost-effectiveness; and cost of pharmacogenomic tests and reimbursement (Jantararoungtong et al., 2021a).

The severity of COVID-19 ranges from being asymptomatic to developing into a fatal acute respiratory syndrome and varies between populations. This can be linked to the variations in the *HLA*. The set of *HLA* alleles might determine the immune responses to viruses according to the selected peptides that can bind to the peptide-binding groove. A recent study from Italy showed an increase in the frequency of *HLA-B*27:07*, *HLA-DRB1*15:01*, and *HLA-DQB1*06:02* among severe COVID-19 patients in a cohort of 99 Italians (Novelli et al., 2020). However, another study from Sardinia in Italy showed a negative influence on the disease course in the presence of the *HLA-DRB1*08:01* allele (Littera et al., 2020). A study from Spain showed that the *HLA-A*11*, *HLA-C*01*, and *HLA-DQB1*04* were associated with higher mortality in a cohort of 72 patients (Lorente et al., 2021). A study from 95 South Asian patients showed an increase in the frequency of *HLA-B*51* and *HLA-DRB1*13* in the fatal group compared to the mild infection group, while increase in the frequency of *HLA-B*35* among the mildly infected group (Naemi et al., 2021). A study in 147 individuals of European descent experiencing variable clinical outcomes following COVID-19 infection showed that a significant difference in the allele frequency of *HLA-DRB1*04:01* was found in the severe patient compared to the asymptomatic group, whereas a significantly lower frequency of the *HLA-DQA1*01:01*, *HLA-DQB1*05:01*, and *HLA-DRB1*01:01* alleles

was found in the asymptomatic group compared to the background population (Langton et al., 2021). A discrepancy between the studies can be attributed to many factors, including sample size and ethnic variations.

In addition to the prediction of certain diseases and certain adverse drug effects, an association between polymorphism in the *HLA* system and COVID-19 severity might have an impact on the implementation of a screening program to identify individuals at risk for COVID-19. In Thailand, the top ranked *HLA* alleles include *HLA-A*11:01* (26.06%), *HLA-B*46:01* (14.04%), *HLA-C*01:02* (17.13%), *HLA-DRB1*12:02* (15.32%), *HLA-DQA1*01:01* (24.89%), and *HLA-DQB1*05:02* (21.28%), and when focusing on *HLA-B*, the most frequent alleles were *HLA-B*46:01* (11.51%), *HLA-B*58:01* (8.62%), *HLA-B*40:01* (8.22%), *HLA-B*15:02* (8.16%), *HLA-B*13:01* (6.95%), and *HLA-B*44:03* (4.21%) (Puangpetch et al., 2014; Satapornpong et al., 2020). According to the study from Spain, Thai people should be alert because *HLA-A*11*and *HLA-C*01* alleles are associated with high mortality. Moreover, Thai population has less frequency of a good prognostic marker such as *HLA-B*35* studied from South Asia. However, clinical analysis of the association between *HLA* allele frequency and COVID-19 severity in Thailand is needed to validate the *HLA* alleles as the appropriate prognostic markers used in Thai clinical practice.

8.2.2 *ACE2*

Angiotensin-converting enzyme-2 (ACE2) is a protein consisting of 805 amino acids encoded by the *ACE2* gene and is expressed in many parts of human cells including oral mucosa and nasopharynx. It is well recognized that ACE2 serves as an entry binding receptor of SARS-CoV-2 through interactions with specific amino acids of this enzyme (Sanders et al., 2020). A recent *in silico* model of possible *ACE2* genetic variants with its interaction with the SARS-CoV-2 spike (S) protein has been analyzed, and it revealed that both rs73635825 (S19P) and rs143936283 (E329G) were shown to interfere with the ACE2 interaction with the S protein of SARS-CoV-2 (Ambrocio-Ortiz et al., 2021). After analyzing SNPs of *ACE2* with susceptibility to SARS-CoV-1 or MERS, recent studies predicted that certain SNPs of *ACE2* should consider COVID-19 patients for assessing the correlation with severity. It was also predicted that COVID-19 severity would vary around the world since the prevalence of the *ACE2* genetic variants was significantly different in various ethnic groups (Benetti et al., 2020; Gemmati and Tisato, 2020; Ambrocio-Ortiz et al., 2021; Bakhshandeh et al., 2021; Biswas, 2021c; Choudhary et al., 2021).

8.2.3 *TMPRSS2*

Transmembrane protease, serine 2 (TMPRSS2) is an enzyme of serine protease family encoded by the *TMPRSS2* gene. During membrane fusion, SARS-CoV-2 "S" protein is activated by the TMPRSS2; therefore, it is postulated that *TMPRSS2* variants might have been correlated to COVID-19 severity. Genetic variants of *TMPRSS2* augmenting TMPRSS2 activity might play an important role in the progression of COVID-19 severity and may be considered as a genetic risk factor (Hou et al., 2020; Choudhary et al., 2021).

9 PHARMACOGENETICS CONSIDERATIONS OF COVID-19 THERAPEUTICS: IMPLICATIONS FOR EFFICACY AND SAFETY

9.1 Antiparasitics

Due to proven ineffectiveness and exclusion from COVID-19 treatment protocols, we have excluded hydroxychloroquine and chloroquine from further analysis in this review.

9.1.1 Ivermectin

Ivermectin underwent extensive metabolism *via* CYP enzymes, predominantly by the CYP3A4 isoform, converting ivermectin into at least 10 metabolites, most of which are hydroxylated and demethylated products (Zeng et al., 1998; González Canga et al., 2008). Ivermectin is also a substrate of P-gp encoded by the *ABCB1*, and genetic polymorphisms of *ABCB1* have linked to severe neurologic ADRs (Lespine et al., 2006; Baudou et al., 2020). Also, ivermectin is transported by the OATP1A2 and OATP2B1 encoded by the *SLCO1A2* and *SLCO2B1*, respectively, although no pharmacogenetic study was identified for this association to date in the literature (Fricke-Galindo and Falfán-Valencia, 2021; Telbisz et al., 2021).

9.2 Antiviral Drugs

9.2.1 Remdesivir

Second, although remdesivir (RDV) is a promising investigational drug proving its activity in cell culture and animal models against SARS-CoV, Middle East respiratory syndrome corona virus (MERS-CoV), and SARS-CoV-2, it is currently not approved for any indication (FDA, 2020). *In vitro* studies suggest that RDV is a substrate for multiple drug metabolizing enzymes, for example, CYP2C8, CYP2D6, and CYP3A4, and also a substrate of OATP1B1 and P-glycoprotein (P-gp) transporters (Takahashi et al., 2020; Deb et al., 2021). Although the pharmacogenomic study of RDV has not been undertaken yet, it is predicted that known variants of these metabolic/transporter genes could affect the safety or efficacy of remdesivir and should assess COVID-19 patients. It is important to note that all of these genes were considered very important pharmacogenes (VIPs) by PharmGKB (Takahashi et al., 2020).

In addition to CYP/transporters' involvement in the PK, RDV prodrug undergoes an intra-cellular sequential metabolism predominantly mediated by hydrolase activity to an active C-adenosine nucleoside triphosphate analog (Eastman et al., 2020; European Medicines Agency, 2020). Upon diffusion of RDV into the cell, the conversion of RDV into the nucleoside monophosphate form is presumably initiated by carboxylesterase (CES)-mediated hydrolysis of the amino acid ester that liberates a carboxylate and then converted to cyclic anhydride (Eastman et al., 2020; McCreary and Pogue, 2020; Yang, 2020). Cyclic anhydride is very unstable and hydrolyzed by water to the alanine metabolite GS-704277 which is further hydrolyzed by phosphoramidase to the nucleoside monophosphate. Nucleoside monophosphate is further phosphorylated in the

presence of nucleoside phosphate kinase enzyme, yielding the active nucleoside triphosphate analog which may act as an analog of adenosine triphosphate (ATP) and competes with the natural ATP substrate to selectively inhibit RNA-dependent RNA polymerase (RdRp). Since the conversion of RDV to pharmacologically active nucleoside triphosphate analog is initiated extensively by the intracellular CES, we predict that genetic variability of *CES* gene-regulating expression may affect the safety or efficacy of RDV, and it is therefore hypothesized that "COVID-19 patients inheriting *CES* genetic polymorphisms might potentially modify efficacy or safety of RDV warranting clinical studies to be assessed for the achievement of precision medicine of RDV."

9.2.2 Ribavirin

A genetic association study of ribavirin in some other viral infections except SARS-CoV-2 revealed that some genetic polymorphisms may result in up to 30% variability of ribavirin trough concentrations, affecting its safety and efficacy. It was found that patients who carried the homozygous variant of the *SLC29A1*, encoding influx transporter, were associated with significantly higher trough concentrations than wild-type variants (2,070 ng/ml vs. 1,837 ng/ml; $p = 0.02$). By contrast, patients who carried the homozygous variant of the *SLC28A2* were associated with significantly lower trough concentrations than wild types (homozygous 1,595 ng/ml vs. heterozygous 1,933 ng/ml vs. wild-type 2,229 ng/ml; $p = 0.04$). This is also consistent with *SLC28A3* variant (homozygous variant 2,294 ng/ml vs. heterozygous variant 1,813 ng/ml; $p = 0.01$) (Allegra et al., 2015). It is also well-established that hemolytic anemia, the most common dose-limiting toxic effect of ribavirin, is protected by various genetic variants of *ITPA*, encoding inosine triphosphatase (D'Avolio et al., 2016). In a meta-analysis of 20 studies, hemoglobin was significantly reduced in patients with wild-type alleles of ITPA compared to the patients having single-nucleotide polymorphisms (SNPs) as reported in a meta-analysis consisting of 20 studies (OR: 12.8, 95% CI: 7.4–22.1 for rs1127354 CC; OR: 3.4, 95% CI: 2.1–5.6 for rs7270101 AA; OR: 4.4, 95% CI: 2.8–7.0 for rs6051702 AA) (Pineda-Tenor et al., 2015). It was established that reduced activity of *ITPA* due to genetic variants governs to the deposition of inosine triphosphate and safeguard against ribavirin-induced very toxic effects, that is, hemolysis. Hemolytic anemia was also found for a short-term use of ribavirin in respiratory viral infection (Burrows et al., 2015). By contrast, a genome-wide association study (GWAS) of 303 patients with hepatitis C viral infection who were administered ribavirin with other therapy showed that the risk of thrombocytopenia was significantly higher in patients with rs6139030 SNP of *ITPA* (OR: 3.9, 95% CI: 2.8–5.5, $p = 1.33 \times 10–15$) (Tanaka et al., 2011). Approximately 25% of the Thai patients carried *ITPA* genetic polymorphisms (Jantararoungtong et al., 2021b), which revealed that considerable proportion of Thai population taking ribavirin would be affected by the *ITPA* genetic variabilitites.

While the association of influx transporter genetic variants or *ITPA* with the safety or efficacy of ribavirin was not investigated in COVID-19 patients, such genetic association assessments are warranted in future clinical studies.

9.2.3 Favipiravir

Favipiravir (FPV) is one of the most potential antiviral drugs currently under considerations in several clinical trials to evaluate its efficacy and safety in patients with COVID-19 (Du and Chen, 2020). The National Medical Products Administration (NMDA) of China approved emergency use of FPV for a clinical trial in adult patients with COVID-19. Being a prodrug, FPV is ribosylated and phosphorylated to form active metabolite called FPV ibofuranosyl-5′-triphosphate, which then competes with purine nucleosides and interferes the viral replication by potentially inhibiting the RdRp of RNA viruses, for example, SARS-CoV-2 (Du and Chen, 2020). FPV is metabolized mainly by aldehyde oxidase and to a less extent by the xanthine oxidase (Takahashi et al., 2020). Although there are no published studies that have specifically assessed the pharmacogenomic influence of FPV, genetic variants of aldehyde oxidase were associated with pharmacodynamic outcomes in other drugs which are substrates of aldehyde oxidase such as azathioprine or allopurinol, and suggesting that PGx of FPV should also be taken into considerations in COVID-19 patients (Takahashi et al., 2020).

9.2.4 Oseltamivir

Oseltamivir is a prodrug which is converted to the active metabolite *via* carboxylesterase 1 (CES1) encoded by the *CES1* (Shi et al., 2006). The SNP rs71647871 of *CES1* has been found to be associated with variation in plasma concentration–time curve of oseltamivir (Tarkiainen et al., 2012) The PK of oseltamivir may also be affected by the *ABCB1*, *CES1*, *NEU2*, and *SLC15A1* genetic variants. The SNP rs1045642 of *ABCB1* was associated with neurologic ADRs developed by oseltamivir (Bermúdez de León et al., 2020; Fricke-Galindo and Falfán-Valencia, 2021).

9.2.5 Nevirapine

Certain genetic polymorphisms of *HLA* and *CYP2B6* may be associated with increased risk of SJS/TEN when treated with nevirapine as reported elsewhere (Martin et al., 2005; Badary, 2021). Also, selective *ABCB1* genetic variants may also be responsible for developing hepatotoxicity as evidenced elsewhere when treated with nevirapine (Vitezica et al., 2008; Badary, 2021).

9.3 Antiretroviral Agents

9.3.1 Lopinavir/Ritonavir

After pharmacogenomic analysis of 1,380 variants in 638 HIV-infected Caucasian patients taking LPV/RTV, four significant variants were identified. LPV/RTV clearance was higher in patients who carried *SLCO1B1*4/*4* homozygous variants and was lower in patients who carried two or more variant alleles of the *SLCO1B1*5*, *ABCC2*, or *CYP3A* tag than in the patients of the reference group (Lubomirov et al., 2010). GWAS after analyzing 290 variants with the toxicity of LPV/RTV among 104 Caucasian patients with HIV revealed that dyslipidemia and hyperbilirubinemia were significantly associated with some genetic variants of the *CETP*, *MCP-1*, *ABCC2*, *LEP*, and

SLCO1B3 genes. Also, a genetic variant of the *IL-6* gene was significantly associated with resulting in diarrhea (all $p < 0.01$) (Aspiroz et al., 2014; Takahashi et al., 2020).

In addition to these, LPV/RTV being a substrate of P-gp was encoded by the *ABCB1* gene. The efficacy and safety of these drug combinations may also be affected by the genetic polymorphisms of *ABCB1* encoding P-gp expression. Over 30% of the Thai patients inherited *C3435T ABCB1* genetic polymorphisms (Sensorn et al., 2013, 2016), suggesting that considerable proportion of Thai population be affected by the *C3435T ABCB1* genetic variant if taking LPV/RTV for combating COVID-19. A recent review hypothesized that the safety or efficacy of LPV/RTV may be affected by the *C3435T* SNP of *ABCB1*, and the risk phenotypes due to carrying this SNP were prevalently highest in Europe (76.8%), followed by America (67%), Asia (63.5%), and Africa (41.4%) (Biswas, 2021b).

9.3.2 Darunavir/Cobicistat

Darunavir being a substrate of CYP3A4 was used simultaneously with cobicistat, a CYP3A4 inhibitor in a clinical trial for COVID-19 for increasing the exposure of darunavir (Takahashi et al., 2020). Genetic variants of *CYP3A4* regulating the function or expression of CYP3A4 may affect the safety or efficacy of darunavir/cobicistat in COVID-19 patients and should be considered in future studies (Takahashi et al., 2020). Although there is no direct evidence that darunavir is a substrate of SLCO3A1, a 12% significantly lower Darunavir clearance was reduced in patients with *SLCO3A1* variant, suggesting that this might be a substrate of darunavir and should assess COVID-19 patients ($p < 0.05$) (Moltó et al., 2013).

9.3.3 Atazanavir

Atazanavir (ATV) is metabolized by UGT1A and is also an inhibitor of CYP3A. Several genetic polymorphisms of *UGT1A1*, for example, *UGT1A1*6*, **28*, **36*, **37*, and **80*, may affect the PK of ATV and may produce toxicity as outlined in the CPIC dosing guidelines. The CPIC pharmacogenomic-based dosing guidelines have recommended to counseling the patients carrying these variants because of possibility for developing severe hyperbilirubinemia (Gammal et al., 2016). A rapid, reliable, cost-effective, and simple assay to detect *UGT1A1* genetic polymorphisms in has already been developed for adoption in routine clinical practice (Sukasem et al., 2016a). The metabolism of ATV is also partially governed by the P-gp encoded by the *ABCB1*, and patients carrying *C3435T ABCB1* SNP may be at risk of hyperbilirubinemia and severe jaundice as well. Numerous studies showed that certain genetic polymorphisms of *APOA5*, *APOC3*, *ABCA1*, and *APOE* genes were associated with increased risk of dyslipidemia in patients taking atazanavir (Zanone Poma et al., 2008; Suwalak et al., 2015; Badary, 2021).

9.3.4 Efavirenz

Since efavirenz is predominantly detoxified by the CYP2B6, therefore, patients may be at increased risk for toxicity such as depression and suicidal tendencies with some *CYP2B6* genetic variants, reducing the function of CYP2B6 (McDonagh et al., 2015; Desta et al., 2019). Pharmacogenomics for this drug have been extensively studied including in Thai HIV patients, and the CPIC guideline has already been developed for guiding patients with CYP2B6 genetic variants (Sukasem et al., 2012; Sukasem et al., 2014a; Manosuthi et al., 2014; Desta et al., 2019). The SNP rs4803419 of *CYP2B6* was independently associated with increased plasma efavirenz concentration as found in a GWAS (Holzinger et al., 2012). Serious toxic effects of efavirenz, for example, depression and suicidal tendencies, can be optimized by adjusting the dose based on *CYP2B6* genotyping results of patients (Desta et al., 2019).

9.4 Interferon β-1b

An interferon (INF) regulatory factor (IRF6) encoded by the *IRF6* was significantly associated with increased risk of liver injury as identified in a case–control study of IFN-β1b-treated multiple sclerosis patients (OR: 8.3, 95% CI: 3.6–19.2; $p = 2.3 \times 10^{-8}$). The results were subsequently confirmed in an independent cohort study of patients with multiple sclerosis in which liver injury was proved with significantly increased aspartate aminotransferase and alkaline phosphatase concentrations for those who carried *IRF6* genetic variants (Kowalec et al., 2018; Takahashi et al., 2020).

9.5 IL-6 and IL-1 Antagonists

Genetic polymorphisms of the *FCGR3A*, *IL6R*, *CD69*, and *GALNT18* genes may affect the efficacy of tocilizumab in RA as reported elsewhere (Maldonado-Montoro et al., 2016; Maldonado-Montoro et al., 2018; Jiménez Morales et al., 2019). It was reported that the *FCGR3A* rs396991TT genotype had a higher response rate at 12 months therapy of tocilizumab in 87 patients with RA (OR: 5.1; 95% CI: 1.2–21.3; $p = 0.03$). Specific Fc fragment of the IgG receptor binding to tocilizumab may be altered by this selective genetic variant and may change systemic clearance of this drug (Jiménez Morales et al., 2019). Polymorphisms of other genes, for example, *IL6R*, *CD69*, and *GALNT18*, have limited direct effects on the safety or efficacy of tocilizumab (Maldonado-Montoro et al., 2016, 2018). Also, relevant pharmacogenomic data affecting either safety or efficacy of other IL-6 or IL-1 antagonists, that is, sarilumab, siltuximab, and anakinra, were not found in the literature (Takahashi et al., 2020). Although considerations of all of these pharmacogene are highly speculative, at least *FCGR3A* rs396991TT SNP should be replicated in COVID-19 patients.

9.6 Inhibitors of the Renin Angiotensin Aldosterone System

Renin angiotensin aldosterone system (RAAS) inhibitors are affected by the *CYP2C9* and *ABCB1* genetic variabilities. For example, patients carrying reduced function alleles of *CYP2C9*, that is, *2, *3, may develop toxicity if taking losartan and dose adjustment based on genotyping of *CYP2C9* could be beneficial to reducing toxicity (Iwamura et al., 2011; Gemmati and Tisato, 2020; Sriram and Insel, 2020; Badary, 2021). Therapeutic response of losartan may also be affected by the *C3435T* SNP

of *ABCB1* since a recent study found a significantly increased absorption of losartan in the early phase in patients who carried this variant (Shin et al., 2020).

9.7 Janus Kinase Inhibitors

Ruxolitinib is metabolized *via* CYP3A4 and CYP2C9, while baricitinib is metabolized partially by CYP3A4 (Umehara et al., 2019; Takahashi et al., 2020; Veeravalli et al., 2020). Both *CYP3A4* and *CYP2C9* genes are tabulated as VIPs in the PharmGKB database, and certain genetic polymorphisms of these genes may affect the safety or efficacy of the, respective, drugs. Also, the PK of baricitinib may be affected by OAT3 transporter encoded by the *SLC22A8* (Takahashi et al., 2020).

9.8 Antibiotics

The PK properties of azithromycin may have interindividual variability due to the variation P-gp expression encoded by the *ABCB1* gene. A single dose of azithromycin had ~2-fold lower peak concentrations for those who carried rs1045642 SNP of *ABCB1* (TT vs. CC: 468.0 vs. 911.2 ng/ml, $p = 0.013$), as found in 20 healthy volunteers (He et al., 2009). It is important to note that genetic variants of *ABCB1* causing increased concentration of azithromycin may be of particular concern when concomitantly used with HCQ/CQ since the additive effects on QT prolongation may exert fatal arrhythmias (Scherrmann, 2020; Takahashi et al., 2020).

9.9 Corticosteroids

Efficacy and toxicities of corticosteroids have been linked to many genetic variants as assessed in various disease conditions. Genes of receptor binding (e.g., *CRHR1* and *NR3C1*), folding proteins (e.g., *ST13*, *STIP1*, and *FKBP5*), metabolic enzymes (e.g., *CYP3A4*, *CYP3A5*, *CYP3A7*, and *GSTT1*), and efflux transporters (e.g., MDR1 and *ABCB1*) may have various genetic polymorphisms accounting for modulating the safety or efficacy of corticosteroids (Song et al., 2017). Pharmacogenetic studies assessing either safety or effectiveness of corticosteroids in either ARDS or COVID-19 were not found in the literature, and it is suggested that the impacts of genetic variants of the genes of interest should focus in future studies in patients with COVID-19 (Takahashi et al., 2020; Vohra et al., 2021).

9.10 Antiplatelets

The effects of *CYP2C19* genetic variants on widely used antiplatelets, for example, clopidogrel in either CAD or stroke patients has been well-established including in Thai patients. The findings of these studies suggest that due to the high risk of major adverse cardiovascular events (MACE) such as death, recurrent MI, stroke, and stent thrombosis for patients carrying *CYP2C19* loss-of-function (LoF) alleles, alternative antiplatelets such as prasugrel or ticagrelor not affected by the *CYP2C19* genetic variants should be prescribed in order to reduce the risk of MACE (Sukasem et al., 2013; Biswas et al., 2020a; Biswas et al., 2021b; Biswas et al., 2022; Biswas and Kali, 2021b; Jafrin et al., 2021). The CPIC dosing guidelines already provided clinical recommendations for clopidogrel in acute coronary syndrome (ACS) patients with *CYP2C19*2*, **3*, **17* variants (Scott et al., 2013). In addition to *CYP2C19* genetic variability, magnitude of P-gp expression regulated by the *ABCB1* genetic variants especially *C3435T* SNP of *ABCB1* may also increase the risk of MACE as established in a recent meta-analysis (Biswas et al., 2020b). The episode of stroke or CAD especially MI is considerably high in severe COVID-19 patients (Bikdeli et al., 2020), and it is generally assumed that antiplatelets, for example, clopidogrel is used in these patients as a supportive care; therefore, it is suggested that pharmacogenomic considerations of antiplatelets are highly desirable to optimize the safety or efficacy of these life-saving drugs in severe COVID-19 patients.

9.11 Anticoagulants

Anticoagulants, for example, warfarin, have wide interindividual response variability due to the presence of *CYP2C9* and *VKORC1* genetic variants as reviewed elsewhere (Jorgensen et al., 2012; Takeuchi et al., 2020). Both the FDA and CPIC have recommended to consider both the *CYP2C9*2*, **3* and *VKORC1* (rs9934438) genetic variants for optimizing its safety, that is, bleeding or efficacy in order to achieve precision medicine of warfarin (Dean, 2012; Johnson et al., 2017). Other new oral anticoagulants such as debigatran, rivaroxaban, and apixaban are affected by the *ABCB1* genetic variants and should be considered clinically for optimizing the safety and efficacy (Xie et al., 2018; Kanuri and Kreutz, 2019).

9.12 Non-Steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs such as celecoxib, flurbiprofen, ibuprofen, and lornoxicam are predominantly metabolized by CYP2C9 and to a lesser extent by CYP1A2 and CYP3A4. Gastrointestinal (GI) bleeding, myocardial infarction, renal damage, *etc*. are the most common serious adverse effects of NSAIDs; however, many NSAIDs are considered safe and are frequently used as the over-the-counter medicine (Theken et al., 2020). A recent meta-analysis showed that individuals with *CYP2C9* poor metabolizers were associated with significantly increased risk of NSAID-related gastrointestinal bleeding (OR: 1.90, $p = 0.003$) and indicated that *CYP2C9*2* was a poor risk predictor, while *CYP2C9*3* was a highly significant predictor of GI bleeding (Macías et al., 2020). The CPIC guidelines provided clinical recommendations based on the *CYP2C9* genotype and suggested to consider *CYP2C9*2* and *CYP2C9*3* variants for patients taking celecoxib, flurbiprofen, ibuprofen, and lornoxicam for optimizing the safety (Theken et al., 2020). Summary of the pharmacogenomics associations of some of the COVID-19 therapeutics with the safety or efficacy is illustrated in **Table 3**.

10 *IN SILICO* PREDICTION OF DRUG EFFECTS IN TREATMENTS FOR COVID-19

To combat COVID-19, computational aided-drug design and screening have been rapidly applied to identify FDA-approved drugs and newly potent compounds from available databases. Using *in silico* approaches, extensive research works have been carried out to acquire an understanding of mechanisms of action

TABLE 4 | Lists of important targets involved in SARS-CoV-2 life cycle mostly used in *in silico* study.

Type		Functions	Important residues	Reference
Host enzymes				
Transmembrane	ACE2	Viral entry	SARS-COV-2 RBD (with corresponding altered residue) Cluster 1 (N-terminus); R439, Q498, N501 Cluster 2 (central); K417, L455, F456, Y473, Q493 Cluster 3 (C-terminus); F486	Xiu et al. (2020)
Viral enzymes				
Proteases	PLpro (Nsp3)	Catalyzes the viral polyproteins	Catalytic residues; C111, H272, and D286	Amin et al. (2021)
	Mpro (Nsp5)	Catalyzes the viral polyproteins	Catalytic residues; H41 and C145	Amin et al. (2021)
RNA-dependent RNA polymerase	RdRp (Nsp12)	Viral RNA synthesis	Catalytic residues; S759, D760, and D761 (motif C)	Ghazwani et al. (2021)

and SARS-CoV-2's activities. However, there are still many foundations to be established for developing novel therapeutics agents for the treatment of COVID-19 (Amin and Jha, 2020). Herein, the current situation in the discovery of anti-SARS-CoV-2 agents at four important targets (**Table 4**) from *in silico* studies is described and summarized as follows.

10.1 Spike Protein

SARS-CoV-2 enters into host cells by transmembrane spike (S) glycoprotein that forms homotrimers protruding from the viral surface. The S glycoprotein consists of two subunits responsible for either host cell receptor binding (S1 subunit including the receptor-binding domain, RBD) or the virus fusion (S2 subunit) (Yang et al., 2020). The ACE2 receptor on the host cell is required for viral entering; however, following entry processes vary depending on the cell type (Xiu et al., 2020). The interface can be divided into three parts by mainly polar and is close to the SARS-CoV-2 S/ACE2 complex (Li et al., 2005; Song et al., 2018). In **Figure 5A**, the extended loop of RBD contacts with ACE2 mainly at the arch-like helix α1 of the proteolytic domain *via* N-terminal, central, and C-terminal (clusters 1–3), and partially at the helix α2 and β loops 3–4 (Xiu et al., 2020). The protein–protein binding is likely found at both terminals: 1) formed hydrogen bonds at the α1 N terminus (cluster 1) between the RBD residues Q498, T500, and N501, and the ACE2 residues Y41, Q42, K353, and R357; and 2) van der Waals interactions of Q474 (RBD)--Q24 (ACE2) and F486 (RBD)--M82 (ACE2) at another end (cluster 3). However, only the residue Y453 from the middle cluster 2 contacts with the ACE2 proteolytic domain at residue H34. The SSAA09E2 from the Maybridge HitFinder small-molecule library can inhibit the S-RBD/ACE2 binding (Adedeji et al., 2013), while the chloroquine (Vincent et al., 2005; Wang et al., 2020a) and hydroxychloroquine (Rainsford et al., 2015) used to treat several human diseases including COVID-19 were found to interfere the ACE2.

From molecular docking study on ~4,000 known drugs from the DrugCentral database (Br et al., 2020) and ~7,000 antiviral agents from the Asinex database (Farouk et al., 2021) on the S/ACE2 interface followed by molecular dynamic (MD) simulation of screened compounds, the glycyrrhizic acid and the compound 6,612 with the highest binding affinity from the two following databases, respectively, were suggested for further *in vitro* and/or *in vivo* tests. The molecular docking, and physicochemical, pharmacokinetic, and MD studies indicated the solanine, acetoside, and rutin from plant-based natural compounds as the S and Mpro dual inhibitors (Teli et al., 2020; Deetanya et al., 2021). Moreover, several natural herbal compounds such as luteolin, andrographolide, zhebeirine, 3-dehydroverticine, ophiopogonin D, glycyrrhizin, saikosaponin C, crocin-1, and militarine formed strong hydrogen bonds at RBD could prevent the viral binding to ACE2 receptor (Stalin et al., 2021). In addition, the peptide antibiotics (polymyxin B, colistin, and daptomycin), pressure regulators (terlipressin and lypressin), hormone peptides (alarelin and leuprorelin), and immunostimulants (thymopentin) able to hamper the RBD/ACE2 interaction were identified by an *in silico* study. Aurintricarboxylic acid and heparin sodium with binding inhibition of 80 and 63% interacted with RBD at clusters 1 and 2, respectively (David et al., 2021). Computational results could help to demonstrate how geraniin can block the viral entry to human cells by preferentially binding at SARS-CoV-2 S RBD in agreement with the biolayer interferometry-based analysis (Kim et al., 2021).

10.2 Proteases

After virion entry into host cells, two polyproteins (pp1a and pp1ab) are translated, which are then divided by two viral proteases: main protease (Mpro) and papain-like protease (PLpro) (Freitas et al., 2020; Goyal and Goyal, 2020). The Mpro, also known as 3C-like protease (3CLpro), has received great attention because of its important involvement in enzymatic activity and post-translational processing of replicase polyproteins. This enzyme has high structural and sequence similarity with SARS-CoV Mpro (Peele et al., 2020; Das et al., 2021b). It contains two catalytic dyad residues C145 and H41 in the active site (**Figure 5B**) (Zhang et al., 2020c), whereas the residues H41, M49, G143, S144, H163, H164, M165, E166, L167, D187, R188, Q189, T190, A191, and Q192 are involved in substrate binding. The hydrophobic side chains are mainly present at the S2 and S4 sites (Amin et al., 2021). **Figure 5C** shows the structure of papain-like protease (PLpro) containing the catalytic triad residues C111, H272, and D286 in the active site. The C111 residue engages in Michael addition to the warhead of inhibitors with a formation of a covalent thioether linkage, while the residues Y268, M208, P247, P248, T301, P248, Y264, N267, Q269, L162,

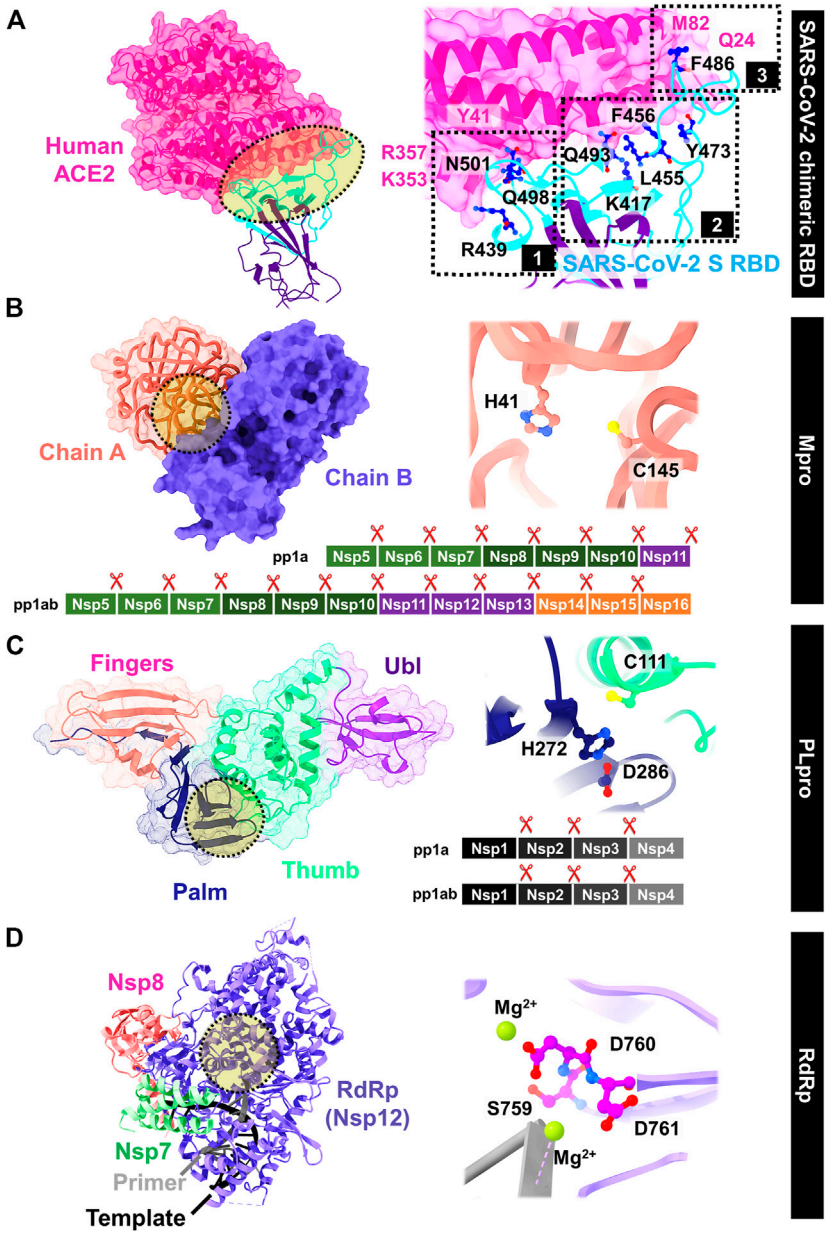

FIGURE 5 | Viral targets for drug development against SARS-CoV-2: **(A)** S RBD/ACE2 binding, **(B)** Mpro, **(C)** PLpro, and **(D)** RdRp, whereas the important residues are also labeled.

C270, G271, and Y273 are involved in substrate binding (Amin et al., 2021).

10.2.1 Main Protease

The *in silico* explorations of potential inhibitors against SARS-CoV-2 Mpro are summarized in **Table 5**. The first crystal structure of the Mpro with covalent inhibitor N3 was reported in January 2020 (Jin et al., 2020), and the co-crystal data available from many other research groups have provided the basis for fast target-based lead drug development against SARS-CoV-2 Mpro. They were utilized to create a pharmacophore model and perform docking research to identify anti-SARS-CoV-2 inhibitors such as lopinavir, remdesivir, ritonavir, saquinavir, and raltegravir (Daoud et al., 2021). Ritonavir was well occupied in the Mpro active site and interacted with the oxyanion hole residues N142 and G143 (Nutho et al., 2020). Jin et al. (2020) screened >10,000 approved drugs, candidates in clinical trials, and pharmacologically active compounds using combined structure-based virtual and high-throughput screening. The two FDA-approved drugs (disulfiram and carmofur) and four clinical trial compounds (ebselen, tideglusib, shikonin, and

TABLE 5 | Inhibitors against SARS-CoV-2 Mpro derived from *in silico* screening.

Type of inhibitor	Name	Computational method for study/screening	Binding affinity prediction (kcal/mol)	Method evaluation	IC_{50} (μM)	Reference
FDA-HIV-drug approved	Lopinavir	MD simulations (AMBER)	−10.89	Cell-based assay (HEK-293 T cell infected SARS-CoV-2)	Indeterminable	(Mahdi et al., 2020; Nutho et al., 2020)
	Ritonavir		−14.93		13.7	
FDA-approved drug Clinical trial compounds	Carmofur	Molecular docking (Glide)	—	Enzyme-based assay	1.82	Jin et al. (2020)
	Disulfiram				9.35	
	Cinanserin				124.93	
	Tideglusib				1.55	
	Ebselen				0.67	
	Shikonin				15.75	
	PX-12				21.39	
SuperDRUG2 database	Binifibrate	E-pharmacophore model-based virtual screening, MD simulations (Desmond module)	−67.78	—	—	Arun et al. (2021)
	Bamifylline		−65.24			
Pfizer company	PF-07321332	MD simulations (Gromacs)	−102.00	—	—	(Ahmad et al., 2021; Baig et al., 2021)
	PF-00835231		—			
DrugBank	DB07800	Pharmacophore- and e-pharmacophore, MD simulations (Gromacs)	−54.04	—	—	T et al. (2021)
	DB03744		−38.83			
	DB03744		−40.25			
	DB02986		−45.01			
	DB03208	Molecular docking (Glide)	−15.07	—	—	Debnath et al. (2021)
	DB03949		−10.91			
	DB08001		−10.15			
	DB08526		−9.30			
	DB02558		−8.94			
	DB12332		−8.78			
	DB02651		−8.70			
FDA-approved drug	Boceprevir	Combined protease pharmacophore clustering and molecular docking (iGEMDOCK)	−25.7	Enzyme-based assay	1.63	Pathak et al. (2021)
				Antiviral activity (Vero cell infected SARS-CoV-2)	49.89 (EC_{50})	
				Cytotoxicity (Vero E6 cell)	159.6 (CC_{50})	
	Telaprevir		−5.5	Enzyme-based assay	11.47	
				Cytotoxicity (Vero E6 cell)	35.8	
Drugs and Probes database	Myricetin	Molecular docking (Glide)	—	Enzyme-based assay (FRET assay)	0.22	Maria et al. (2021)
	Thioguanosine				6.3	
	MG-132				7.4	
	Bronopol				0.4	
	Sennoside A				1.59	
	ML311				0.15	
	PR-619				0.41	
	Felbinac ethyl				0.20	
	Z-DEVD-FMK				0.01	
	Oltipraz				0.21	
Natural drugs from ZINC database	Daidzin	Pharmacophore mapping and MolDock	−115.11	—	—	Saeed et al. (2021)
Peptidomimetic	N3	MD simulations (AMBER)	−9.92	Cell-based assay (qRT–PCR in Vero cell infected SARS-CoV-2)	16.77	(Jin et al., 2020; Amin et al., 2021; Somboon et al., 2021)
	11a		−9.68	Enzyme-based assay	0.053	
	13b		−10.35	Enzyme-based assay	0.67	
	14b		−9.64	—	—	
Anthocyanin-derived compounds from the PubChem database	ID44256891	Structure-based pharmacophore modeling PHASE module and molecular docking (Glide)	−12.37	—	—	Fakhar et al. (2021)
	ID 44256921		−11.59			
	ID102452140		−10.94			
	ID131751762		−10.30			
	ID131831710		−13.59			
	ID139031086		−9.58			

(Continued on following page)

TABLE 5 | (*Continued*) Inhibitors against SARS-CoV-2 Mpro derived from *in silico* screening.

Type of inhibitor	Name	Computational method for study/screening	Binding affinity prediction (kcal/mol)	Method evaluation	IC_{50} (μM)	Reference
N-phenyl-2-(pyrimidin-2-ylthio)acetamide analogs	N-(3,4-dichlorophenyl)-2-(5-(2-phenylthiazol-4-yl) pyrimidin-2-ylthio) acetamide	Virtual screening and 3D-QSAR	—	Edans-Dabcyl FRET assay	3.0	Tsai et al. (2006)
MolPort compound library	M-8524	Combined virtual screening, MD simulations, and machine learning	—	Enzyme-based assay (FRET assay)	31.0	Glaab et al. (2021)

PX-12) showed potent SARS-CoV-2 Mpro inhibition with the IC_{50} range of 0.67–21.4 μM. Using the active site conformations of SARS-CoV-2 Mpro through protease pharmacophore clustering, the resulting anti-HCV drugs boceprevir and telaprevir and the anti-HIV drug nelfinavir from a set of 2,122 drugs exhibited significant Mpro inhibition and antiviral efficacy in the micromolar range (Pathak et al., 2021). From the superDRUG2 database, binifibrate and bamifylline identified by e-pharmacophore modeling using the Mpro structure co-crystalized with imidazole–carboxamide inhibitor can bind tightly at the active site and form hydrogen bonds with G143 and E166 throughout MD simulation (Arun et al., 2021). For the mechanism of action for the drug candidates against SARS-CoV-2 Mpro currently studied in clinical trials, the dynamic behavior of PF-07321332 and PF-00835231 showed hydrogen bond formations with C145, E166, and Q189 residues, while additional hydrogen bonds with G143 and H164 were observed in PF-00835231 binding (Ahmad et al., 2021; Baig et al., 2021).

Some natural products with promising pharmacodynamic and pharmacokinetic characteristics, for example, higenamine hydrochloride, phloretin, daidzin, and naringenin chalcone, were screened from the ZINC database using the receptor-based pharmacophore modeling and molecular docking (Saeed et al., 2021). The receptor-, ligand-, and machine learning-based screening methods elucidated the small-molecule inhibitors of Mpro with IC_{50} in the micromolar range: rottlerin (37 μM), amentoflavone (143 μM), baicalein (208 μM), and synthetic compounds (e.g., CID 46897844, 31 μM) (Glaab et al., 2021). The crucial residues frequently participating within these potent compounds are E166, T190, Q189, and Q192, while the catalytic residues H41 and C145 are important for amentoflavone and baicalein, respectively. The inhibitors such as N3 and myricetin which covalently bound to C145 could terminate the SARS-CoV-2 Mpro functions (Jin et al., 2020; Glaab et al., 2021). The MD study on Mpro in complex with the four reported peptidomimetic inhibitors N3, 11a, 13b, and 14b indicated that the ligand–protein complexation is mainly driven by vdW and hydrogen bond interactions (Somboon et al., 2021). The polar moieties (e.g., benzamide) and the bulky N-terminal protecting groups (e.g., thiazole) should be introduced to P1' and P4 sites of 13b structure to increase hydrogen bonds and hydrophobic interactions, respectively.

10.2.2 Papain-Like Protease

The two irreversible inhibitors VIR250 and VIR251 with a significant degree of SARS-CoV-2 PLpro selectivity over other proteases were discovered (Rut et al., 2020), and their crystal structures were widely used for virtual screening. The PLpro inhibitors derived from *in silico* screening are illustrated in **Table 6**. The antidiabetic drug phenformin, anti-HIV drug ritonavir, and natural compound quercetin resulted from ~1,700 clinical FDA-approved drugs showed favorable pharmacokinetics and strong binding interactions with SARS-CoV-2 PLpro (Kandeel et al., 2021). In addition to quercetin, PLpro has also been shown to bind with several compounds from the 26 Chinese herbal medicines such as cryptotanshinone and tanshinone IIa (Zhang et al., 2020b). Some anti-HCV drugs, namely, simeprevir, grazoprevir, and vaniprevir, with PLpro inhibition synergize with remdesivir to reduce SARs-CoV-2 virus replication in Vero and/or human cells (Bafna et al., 2021).

The naphthalene-based derivatives with previously reported SARS-CoV-1 PLpro inhibitory activity could be beneficial for SARS-CoV-2 treatment (IC_{50} values of 2.4 and 5 μM for GRL-0617 and compound 6, respectively) due to almost identical residues in the PLpro BL2 loop of the two viruses (Amin et al., 2021). The selective compounds from the ENAMINE REAL database using pharmacophore modeling which have IC_{50} values of 159–505 nM bind to the target protein in a similar manner to the non-covalent SARS-CoV-2 PLpro inhibitor, GRL-0617 (Stasiulewicz et al., 2021). The identified deubiquitinase inhibitors against PLpro, TCID and DUB-IN-3 with IC_{50} of 6.42 and 12.5 μM, formed hydrogen bonding with the PLpro residues Y264 and R166, respectively (Liu et al., 2021). The *in silico* molecular interaction-based method was used to elucidate the cyanobacterial metabolites against SARS-CoV-2 PLpro. The deoxycylindrospermopsin binding with the important residues T26, C44, F140, S144, C145, H163, and E166 was identified as the most promising inhibitory candidate (Naidoo et al., 2021). By molecular docking and MD study of 97 antiviral secondary metabolites from fungi, norquinadoline A was found to be the most effective inhibitor of SARS-CoV-2 PLpro with high gastrointestinal absorption, low

TABLE 6 | Inhibitors against SARS-CoV-2 PLpro derived from *in silico* screening.

Type of inhibitor	Name	Computational method for study/screening	Binding affinity prediction (kcal/mol)	Method evaluation	IC_{50} (μM)	Reference
FDA-approved drug	Phenformin	MD simulations (AMBER)	−56.5	–	–	Kandeel et al. (2021)
	Quercetin		−40.9			
	Ritonavir		−37.6			
	Montelukast		−36.4			
	Fostamatinib 1		−33.5			
	Candesartan		−28.9			
Chinese herbal medicine	Cryptotanshinone	Molecular docking (AutoDock Vina)	−5.25	–	–	Zhang et al. (2020b)
	Tanshinone IIa		−5.02			
PubChem and ZINC databases	Deoxycylindrospermopsin	MD simulations (Gromacs)	−41.39	–	–	Naidoo et al. (2021)
ENAMINE REAL database	rac3j	Pharmacophore model-based virtual screening	–	Fluorescence polarization-based PLpro activity Enzyme-based assay	1.40	(Freitas et al., 2020; Klemm et al., 2020; Stasiulewicz et al., 2021)
	rac3k				1.15	
	rac5c				0.81	
	6577871				100.7	
	7724772				23.5	
	Compound 6				5.0	
Antiviral secondary metabolites from fungi	Norquinadoline A	Molecular docking (UCSF	−10.9			Quimque et al. (2021)
	Asperterrestide A		−8.9			
	Rubrolide S		−8.7			
	Isoaspulvinone		−7.7			
	Deoxynortryptoquivaline		−9.6			
	Arisugacin A		−10.0			
	Isochaetochromin D1		−9.9			
	Penicillixanthone A		−9.5			

blood–brain barrier penetrability, and high drug-likeness (Quimque et al., 2021).

10.4 RNA-Dependent RNA Polymerase

RNA-dependent RNA polymerase or RdRp (Nsp12) catalyzes viral RNA synthesis, and as a result, it plays a key role in viral replication and multiplication, alongside cofactors Nsp7 and Nsp8 proteins. Among seven key motifs in RdRp catalytic domain, motifs A–F are highly conserved across all viral RdRps, but motif G is a unique structural characteristic of primer-dependent RdRps in some positive-sense RNA viruses binding with the primer strand at the beginning of RNA synthesis. In **Figure 5D**, the catalytic residues S759, D760, and D761 with Mg^{2+} as a catalyst cofactor are located in motif C (Naydenova et al., 2021), while the other crucial residues are D618, C622, and D623 in the active site, S682, T687, A688, and N691 in motif B, and K545, R553, and R555 in motif F (Ghazwani et al., 2021).

Favipiravir is the first antiviral drug authorized for the treatment of SARS-CoV-2 by China's National Medical Products Administration. Several drugs, that is, sofosbuvir, ribavirin, galidesivir, and remdesivir, are being tested in clinical trials against SARS-CoV-2 RdRp (Elfiky, 2020). In the reported crystal structures, the drugs favipiravir and remdesivir are accommodated in the ATP binding site of RdRp (Yin et al., 2020; Naydenova et al., 2021). *In silico* drug design and discovery of RdRp inhibitors are given in **Table 7**. In addition to remdesivir and ribavirin, the molecular docking study of 1,749 antiviral drugs suggested that paritaprevir, glecaprevir, and velpatasvir also showed interesting interactions with RdRp (Singh et al., 2021). The top 50 compounds retrieved from structure-based virtual screening of 15,220 compounds from DrugBank and TargetMol Bioactive compounds Library against SARS-CoV-2 RdRp were evaluated by bio-layer interferometry (BLI) binding followed by cell-based polymerase activity assays (Li et al., 2021). Corilagin showed the highest inhibition SARS-CoV-2 RdRp (K_D of 0.54 220 μM) and inhibited viral replication in Vero cells (EC_{50} of 0.13 μM), by binding at RdRp's palm domain and thus preventing the conformational changes necessary for nucleotide incorporation. Its binding pocket comprised the conserved residues S759, D760, and D761 in motif C, and the surrounding residues G616, D761, K798, W61, W800, D618, S814, E811, S549, C799, and A550. Relative to remdesivir, the 11 obtained compounds from the virtual screening and MD simulations derived from the ZINC database displayed significant interactions with all RdRp active site residues (Ghazwani et al., 2021). Based on pharmacophore modeling of the remdesivir/RdRp complex (two anionic acceptor, one donor, one acceptor, and one dual donor and acceptor features), the epigallocatechin gallate, kuromanin, procyanidin-b-2, and rutin were the top four hits among 5,836 compounds from the ChEMBL database (Kandwal and Fayne, 2020). Pharmacophore modeling, structure-based virtual screening, and MD simulations of RdRp bound with the known RdRp inhibitors were used to screen the potential agents from the six databases; PubChem-134297651, CHEMBL387201, CHEMBL1196124, PubChem-122704503, ZINC257357489, and ZINC5605331,

TABLE 7 | Inhibitors against SARS-CoV-2 RdRp derived from *in silico* screening.

Type of inhibitor	Name	Computational method for study/screening	Binding affinity prediction (kcal/mol)	Method evaluation	IC_{50} (μM)	Reference
DrugBank and TargetMol Bioactive compounds Library	Verbascoside	Molecular docking (AutoDockTools), MD simulations (AMBER)	–8.3	Bio-layer interferometry (BLI) binding assay	0.84 (K_D)	Li et al. (2021)
	Oleanonic acid		–9.6		171.00	
	Forsythoside A		–8.7		1.61	
	MK-3903		–8.9		220.00	
	T-5224		–8.6		38.70	
	Corilagin		–8.9		0.54	
				Cell-based assay (Vero cell infected SARS-CoV-2)	0.13 (EC_{50})	
				Cell-based assay (Huh-7 cell infected HCoV-OC43)	2.49 (EC_{50})	
	Remdesivir		—	Cell-based assay (Vero cell infected SARS-CoV-2)	0.06 (EC_{50})	
				Cell-based assay (Huh-7 cell infected HCoV-OC43)	4.96 (EC_{50})	
ZINC Drug Database and DrugBank	Favipiravir	Virtual screening and molecular docking (Glide; induced-fit docking)	–4.8	Cell-based assay (Vero cell infected SARS-CoV-2)	22.50 (EC_{50}) >100 μM (CC_{50})	(Wang et al., 2020a; Poustforoosh et al., 2021)
	Ribavirin		–6.9		109.5 (EC_{50}) >400 μM (CC_{50})	
	Galidesivir		–7.1	—	—	
	Tenofovir		–5.7			
	Valganciclovir		–5.0			
	Ceftibuten		–5.1			
	Fenoterol		–7.5			
	Silybin		–6.9			
	Idarubicin		–7.9			
Approved small-molecule drugs	Dexamethasone metasulfobenzoate	Molecular docking (AutoDock VINA)	–8.7	—	—	(Jeon et al., 2020; Khater et al., 2021)
	Conivaptan		–8.6	Vero cell infected SARS-CoV-2	10	
	Dutasteride		–8.6	—	—	
	Hesperidin		–8.6			
	Lumacaftor		–8.6			
	Glycyrrhizic acid		–8.6			
	Dexamethasone metasulfobenzoate		–8.6			
	Ergotamine		–8.5	Predicted IC_{50}	190	
	Eltrombopag		–8.5	Vero cell infected SARS-CoV-2	8.0 >50 μM (CC_{50})	
	Astemizole		–8.4	—	—	
	Chlorhexidine		–8.4			
	Gliquidone		–8.4			
ChEMBL, Chem Div, Molport, the NCI open Chemical repository, PubChem, ZINC purchasable databases	PubChem-134297651	Pharmacophore-based virtual screening (HipHop algorithm) and molecular docking (Gold)	72.61 (Gold score)	—	—	Fayyazi et al. (2021)
	CHEMBL387201		64.85 (Gold score)			
	CHEMBL1196124		64.34 (Gold score)			
	PubChem-122704503		61.25 (Gold score)			
	ZINC257357489		47.44 (Gold score)			
	ZINC5605331		40.29 (Gold score)			

(Continued on following page)

TABLE 7 | (*Continued*) Inhibitors against SARS-CoV-2 RdRp derived from *in silico* screening.

Type of inhibitor	Name	Computational method for study/screening	Binding affinity prediction (kcal/mol)	Method evaluation	IC_{50} (μM)	Reference
	MAW-22	Fragment-based drug design, docking, MD simulations (Gromacs)	–390.24			El Hassab et al. (2020)
ChEMBL database	Epigallocatechin gallate Kuromanin Procyanidin-b-2 Rutin	Pharmacophore-based virtual screening (MOE Module)	—	—	—	Kandwal and Fayne, (2020)

which were highly interacting with RdRp at the ATP binding pocket (Grzybowski et al., 2002).

11 PHARMACOGENOMICS FOR COVID-19 VACCINE

At least 13 different vaccines have been administered until now to combat the biggest infectious challenges of the 21st century. Although majority of these vaccines are well-tolerated, they may not be responsive similarly to everyone and may also account for some vaccine-related side/toxic effects. The application of existing pharmacogenetic/pharmacogenomic science to vaccines is termed as "vaccinomics" (Hoffman et al., 1998; Poland et al., 2007, 2021; Omersel and Karas Kuželički, 2020; Soiza et al., 2021). Since the pharmacogenomic association with the safety or efficacy of many clinical important medications, for example, antiepileptics, antiplatelets, cardiovascular drugs, antidepressants, and anticancers, have been well-established and many of these considerations are now implemented in routine clinical practice in some parts of the world, we are expecting that similar approaches in terms of COVID-19 vaccines will start to appear very soon.

Some genetic insights for vaccines applied in other infectious diseases have already been recognized, for example, specific genetic polymorphisms of the *TLR3* gene were associated with significantly reduced immune responses to the measles vaccine as reviewed elsewhere (Poland et al., 2018). Vaccinomics provide a promising newly evolving research area through which the safety or efficacy of vaccines may be optimized. A wide range of genotype/phenotype association data are currently being integrated into this newly emerging research field for many live viral vaccines and expecting that similar attributes will begin for COVID-19 vaccines as well. The application of vaccinomics in COVID-19 may allow us to explain the interindividual immune responses' variability and adverse events, and may also accelerate the development of personalized vaccine (Poland et al., 2021).

Immunogenicity data of COVID-19 vaccination come from the assessment of specific T-cell responses and specific antibody responses (Zhu et al., 2020). Both activation of cytotoxic T cell and antibody production need antigen presentation *via* HLA class I and II, respectively. We hypothesize that variation of the *HLA* genotype might affect the immunogenicity of COVID-19 vaccines. Further investigation in association between *HLA* polymorphism and COVID-19 vaccine immunogenicity is interesting and might help to predict individual COVID-19 vaccine effectiveness.

Within a year, several vaccines have been developed and millions of doses were delivered. The ChAdOx1 nCoV-19 vaccine (AstraZeneca) has recently been reported as an increasing risk of venous thrombosis and thrombocytopenia, called vaccine-induced immune thrombotic thrombocytopenia (VITT), 7–10 days after receiving the first dose (Schultz et al., 2021). Recently, some studies showed that pathogenic antibodies to platelet factor 4 (PF4), which have a major role to develop VITT, can occur after the administration of the ChAdOx1 nCoV-19 vaccine (Scully et al., 2021). This pathogenic PF4-dependent syndrome is unrelated to the use of heparin therapy, called heparin-induced thrombocytopenia (HIT). The higher PF4 level has an association with *HLA-DRB1*03:01-DQB1*02:01* haplotype (Zhang et al., 2019). Genetic variants of *HLA-DRB1* and *HLA-DQB1* were found ~25% in Thai population (Puangpetch et al., 2014; Satapornpong et al., 2020), indicating that considerable proprotion of Thai population might be at risk of developing VITT associated with these genetic polymorphisms. Clinical validation of the screening of *HLA-DRB1*03:01* and *HLA-DQB1*02:01* to predict the occurrence of VITT should be investigated, and it might help people to avoid this life-threatening condition. Even though platelet level is very low, VITT should not be treated by platelet transfusion. Ideally, such transfusion should be avoid because it would provide a substrate for further antibody-mediated platelet activation and coagulopathy. Therefore, rapid recognition of VITT is very important.

12 PRECISION MEDICINE FOR COVID-19 TREATMENT: KEY CLINICAL CONSIDERATIONS

12.1 Drug–Drug Interactions

It is also alarming that many patients are developing serious complications, for example, life-threatening adverse drug reactions. Since the mortality of COVID-19 patients is significantly higher in older patients and also for those having multiple comorbidities (Biswas et al., 2021a), it is likely to expect DDIs and consequently ADRs generated from the risk of polypharmacy, as evidenced in recent observational studies (Ramírez et al., 2020; Falcão et al., 2021). For example, at least 62 life-threatening potential DDIs of LPV/RTV that should be

TABLE 8 | Potential clinically significant DDIs of COVID-19 therapeutics.

COVID-19 therapeutics	Interacting drug/herb/fruits	Effects of DDI/DHI	Reference
Ribavirin	Warfarin	Decrease anticoagulant effects of warfarin	Rezaee et al. (2021)
Ribavirin	Azathioprine (AZA)	Increase risk of f myelotoxicity, that is, anemia, and thrombocytopenia of AZA	Rezaee et al. (2021)
Lopinavir/ritonavir	Amiodarone Dronedarone Flecainide Ivabradine Propafenone Mexiletine	Cardiac toxicity and QT prolongation	Rezaee et al. (2021)
Lopinavir/ritonavir	Rivaroxaban	Risk of bleeding	Rezaee et al. (2021)
Lopinavir/ritonavir	Simvastatin	Risk of rhabdomyolysis	Rezaee et al. (2021)
Lopinavir	Alfuzosin	Increase QT interval	Rezaee et al. (2021)
Lopinavir/ritonavir	Rifampicin	Increase risk of hepatocellular toxicity	Rezaee et al. (2021)
Lopinavir/ritonavir	Salmeterol	Increase cardiac complications	Rezaee et al. (2021)
Tocilizumab	Dabigatran and etexilate	Risk of thrombosis	Rezaee et al. (2021)
Tocilizumab	Adalimumab	Risk of serious infection and immunosuppressive effects	Rezaee et al. (2021)
Remdesivir	Rifampicin	Increase the risk of hepatotoxicity	Rezaee et al. (2021)

considered in COVID-19 patients if taking these drugs have been identified (Biswas, 2020). A summary of very important clinically significant DDIs of COVID-19 therapies as evidenced or suggested elsewhere is shown in **Table 8**.

DDIs are likely important in case of assessing pharmacogenomic effects of any particular drug since sometimes the DDI may alter/exacerbate the clinical effects or change phenotypes (phenoconversion) associated with interactions with the, respective, genes (Klomp et al., 2020). A recent systematic review reported a phenoconversion from a higher metabolizer phenotype into a lower metabolizer phenotype by the concurrent use of CYP inhibiting drugs and also by the extrinsic factors such as cancer, inflammation, and older age. By contrast, phenoconversion from a lower metabolizer phenotype into a higher metabolizer phenotype was reported by the concomitant use of CYP inducer drugs and also by smoking. In addition, alcohol, pregnancy, and vitamin D exposure may also contribute to the phenoconversion process (Klomp et al., 2020).

If any patients are taking COVID-19 therapeutics influenced by the CYP metabolism and taking CYP inhibitors, also carrying *CYP* genetic variants, then the net clinical effects may be further exacerbated profoundly. There is evidence for such phenomenon as reported in a recent meta-analysis showing that for patients carrying *CYP2C19* LoF alleles and taking clopidogrel and proton pump inhibitors, the risk of MACE was almost over double compared to the patients taking clopidogrel with or without *CYP2C19* LoF alleles (Biswas et al., 2021b). Therefore, DDIs are very important clinical considerations for optimizing safety or effectiveness of COVID-19 medications through assessing pharmacogenomic interventions.

12.2 Patients' Condition and Underlying Diseases

Systemic vascular inflammation and coagulopathy resulting from cytokine storm contribute to multi-organ failure in patients with severe COVID-19. In addition to the genetic biomarker, that is, *HLA* genotype, the non-genetic biomarkers associated with the severity and disease progression of COVID-19 can be divided into 1) hematological biomarkers [lymphocyte count, neutrophil count, and neutrophil–lymphocyte ratio (NLR)], 2) inflammatory biomarkers [C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and procalcitonin (PCT)], 3) immunological biomarkers (IL-6 and IL-10), 4) biochemical biomarkers [D-dimer, troponin, creatine kinase (CK), and aspartate aminotransferase (AST)], and 5) new laboratory biomarkers (homocysteine and angiotensin II) (Ponti et al., 2020a). Almost all these biomarkers are previously used to monitor the critically ill patients with systemic infection/inflammation or multi-organ failure from several causes.

The levels of cytokine storm are associated with COVID-19 severity and severe progression. Among them, IL-6 and IL-10 could be used as biomarkers for fast diagnosis of patients with a higher risk of disease deterioration (Han et al., 2020). The identification of IL-6 might also potentially benefit from anti-IL-6 immunotherapies with tocilizumab (Zhang et al., 2020a).

The level of D-dimer or fibrin degradation products (FDPs) is a strong evidence of thrombosis and thromboembolism (Halaby et al., 2015). Studies have reported an increase in D-dimer and fibrinogen concentrations in the early stages of COVID-19 disease; a 3 to 4-fold rise in D-dimer levels is linked to poor prognosis (Rostami and Mansouritorghabeh, 2020). Monitoring the level of D-dimer might help in determining the prognosis of patients and making the decision of early aggressive treatment.

Plasma levels of homocysteine are associated with vascular inflammation and damage (Balint et al., 2020). Recent data demonstrated a predictive value of homocysteine for the severity of pneumonia from COVID-19 (Ponti et al., 2020b). The high plasma level of Ang II also has been demonstrated in COVID-19 patients with severe lung injury and high viral load (Gheblawi et al., 2020; Liu et al., 2020). When we get further clinical validation, these two new biomarkers would be useful to predict or determine the severity of pneumonia in COVID-19 patients.

12.3 Drug–Herb Interactions

Medicinal plants often serve as a very crucial alternative or adjuvant therapy to synthetic allopathic drugs for combating numerous diseases from the ancient time. Andrographolide

TABLE 9 | Potential DHIs of COVID-19 therapeutics and herbs.

COVID-19 therapeutics	Interacting herbs/components	Effects of DHIs	Involvement of CYP enzymes	Reference
Lopinavir/ritonavir	Qingfei Paidu decoction (QPD) consisting of 20 herbs	*In vivo* study revealed that QPD extended the half-life of lopinavir by 1.40-fold and raised the AUC by 2.04-fold	Through strong inhibition of CYP3A	Zhang et al. (2021a)
Lopinavir/ritonavir	*Echinacea purpurea*	Although PK of these combination drugs was not affected significantly, it may affect when using CYP3A4 substrate drugs	Induce CYP3A	Penzak et al. (2010)
Lopinavir	*Ginkgo biloba*	May reduce drug exposure	Induce CYP3A	Robertson et al. (2008)
Efavirenz and nevirapine	*Hyptis suaveolens*, *Myrothamnus flabellifolius*, *Launaea taraxacifolia*, and *Boerhavia diffusa*	Risk of drug toxicity	Inhibit CYP2B6	Thomford et al. (2016)
Nevirapine	*Hypericum perforatum*	Increase plasma concentration of nevirapine	Induce CYP2B6	Chen et al. (2012)
Atazanavir	*Astragalus membranaceus*	Weak herb–drug interaction	Weak inhibition of CYP3A2	Cheng et al. (2015)
Darunavir/ritonavir	*Echinacea purpurea*	Decrease darunavir concentration	Induce CYP3A4	Moltó et al. (2011)
Losartan	*Silymarin*	Reduce metabolism	Inhibit CYP2C9	(Han et al., 2009; Rombolà et al., 2020)
Indinavir	*Hypericum perforatum*	Decrease plasma concentration of indinavir by 57%	Induce CYP3A4	Chen et al. (2012)
Lopinavir	HEJG consisting of nine herbs	Significantly increase the plasma level of lopinavir by 2.43-fold	Inhibit CYP3A	Zhang et al. (2021b)
Warfarin	*Andrographis paniculata*	Increase systematic exposure of warfarin	Inhibit CYP2C9, CYP3A4	(Pan et al., 2011; Zhang et al., 2018)
	Ginkgo biloba	Increase bleeding risk	Inhibit CYP2C9	(Chen et al., 2012; Asher et al., 2017)
Clopidogrel	St John's wort	Increase responses of clopidogrel	Induce CYP2C19	Rahimi and Abdollahi, (2012)
	Ginkgo biloba	Increase risk of bleeding	Through CYP2C19	(Aruna and Naidu, 2007; Deng et al., 2016; Asher et al., 2017)

Here, AUC, area under the concentration–time curve.

isolated form the medicinal plant of *Andrographis paniculata* has been clinically used for the treatment of inflammatory diseases and viral infections for many years. Recent molecular docking suggests that andrographolide is able to form a covalent bond with the active site of SARS-CoV-2 and may suppress the progression of this pandemic virus. This herbal component may therefore serve as an alternative option for the management of the COVID-19 pandemic (Shi et al., 2020b).

However, interaction of andrographolide with other drugs called drug–herb interactions (DHIs) might be of particular interest in assessing its potency against SARS-CoV-2 infection. An *in vitro* study found potent inhibitory effects on the activities of CYP3A4 and CYP2C9 enzymes by andrographolide, suggesting that drug–herb interactions (DHIs) of andrographolide would be of particular concern for drugs primarily metabolized by CYP3A4 and CYP2C9 pathways such as warfarin (Pan et al., 2011). This is consistent with the findings of another study suggesting that andrographolide could cause DHIs in humans through interfering of CYP2C9 or CYP3A4 enzyme activities (Pekthong et al., 2008, 2009). DHI between andrographolide and warfarin has already been established in the mouse model where andrographolide was found to increase the systemic exposure of warfarin probably by the inhibition of the CYP3A4- or CYP2C9-mediated warfarin metabolism (Zhang et al., 2018). Another study found a DHI between andrographolide and tolbutamide where andrographolide enhanced the metabolic rate of tolbutamide by increasing the expression and activity of certain CYP enzymes (Chen et al., 2013). Also, andrographolide could also induce CYP1A1 and CYP1A2 expression as found in the mouse model (Jaruchotikamol et al., 2007), potentially interacting with drugs metabolized by CYP1A1 or CYP1A2. Many other herbs may also interact with COVID-19 therapies as shown in **Table 9**.

In addition of DHIs, we are also concerned about the pharmacogenetics of herbs termed as "herbogenomics," potentially affecting the safety or efficacy of herbs. For examples, genetic polymorphisms of *CYP3A4* or *CYP2C9* may modify the clinical effects of andrographolide and should be considered clinically along with DHIs. Although the concept is new, it suggests to considering herbogenomics in future studies for patients taking COVID-19 medications. In addition to andrographolide, many other herbs are using against SARS-CoV-2 infection, as shown in **Table 10**.

Because of the antiviral activity of curcumin against various viruses, for example, HIV, Zika virus, herpes simplex virus, Chikungunya virus, hepatitis viruses, and adenovirus (Prasad and Tyagi, 2015; Mounce et al., 2017; Praditya et al., 2019), it may be potentially applied in the management of COVID-19 patients (Ho et al., 2021). A recent molecular docking study revealed that curcumin may inhibit the host entry of SARS-CoV-2 by interfering the viral S protein and host ACE2 receptor protein (Das et al., 2021b). Also, the existing antithrombotic,

TABLE 10 | Herbs using against COVID-19 management.

Herb name/scientific name	Active herb components/whole herbs	Potential mechanism of action against SARS-CoV-2	Reference
Andrographis paniculate	Andrographolide	Forms a covalent bond with the active site of SARS-CoV-2 and may suppress the progression as found in a recent molecular docking study	Shi et al. (2020b)
Turmeric (*Curcuma longa*)	Curcumin	Inhibits the host entry of SARS-CoV-2 by interfering viral S protein and host ACE2 receptor protein	Das et al. (2021a)
Eucalyptus globulus *Corymbia citriodora*	Citronellol, alpha-terpineol, *o*-cymene, d-limonene, eucalyptol, alpha-pinene, and 3-carene	*In silico* study reported potential inhibitor of SARS-CoV-2 M^{pro}	Panikar et al. (2021)
Garlic (*Allium sativum*)	Allicin and allitridin	Interacts with SARS-CoV-2 M^{pro} protease	(Donma and Donma, 2020; Rouf et al., 2020)
Houttuynia cordata	Alkaloids, polyphenols, and flavonoids	Inhibits RdRp	(Das et al., 2021a; Bahadur Gurung et al., 2021)
Ginger (*Zingiber officinale*)	24-Methylcholesta-7-en-3β-on, spinasterone, and spinasterol	Inhibits SARS-CoV-2 3CL protease enzyme	Zubair et al. (2021)
Strawberry (*Fragaria ananassa* Duch.)	With silver nanoparticle (AgNPs)	Demonstrated marked activity against SARS-CoV-2	Al-Sanea et al. (2021)
Ginger (*Zingiber officinal*)	Neohesperidin	*In silico* study demonstrated that neohesperidin potentially binds to both human AAK1 protein and SARS-CoV-2 NSP16 protein	
Andrographis paniculata	Diterpene, flavonoids, and aglycone/glycoside	Inhibits SARS-CoV-2 M^{pro} protease	Sukardiman et al. (2020)
Licorice or Glycyrrhizae (GR)	Flavonoids and terpenes/saponins	May modify TNF and IL-17 signaling pathways, and helps to overcome SARS-CoV-2 infection	Ng et al. (2021)
Licorice (*Glycyrrhiza glabra*)	Glycyrrhizin (GR) and glycyrrhetinic acid (GA)	GR can interfere with virus entry by directly interacting with ACE2 and spike	Diomede et al. (2021)
Siparuna cristata	Retusin and kumatakenin	*In silico* found inhibitory effect against 3CLpro and PLpro SARS-CoV-2 protease	Leal et al. (2021)
Reynoutria Rhizomes	Procyanidins and anthranoids	Strong inhibitor of SARS-CoV-2 M^{pro}	Nawrot-Hadzik et al. (2021)
Ashwaganha *Withania somnifera* *Tinospora cordifolia* (giloy) *Ocimum sanctum* (tulsi)	Withanoside V, somniferine tinocordiside, vicenin, isorientin 40-O-glucoside 200-O-*p*-hydroxybenzoagte, and ursolic acid	These phytochemicals bind with SARS-CoV-2 M^{pro}	Shree et al. (2020)
Allium sativum (garlic)	Allicin and allitridin	Interacts with the M^{pro} protease	(Donma and Donma, 2020; Rouf et al., 2020)
Vitex negundo and *Justicia adhatoda*	Eudesmol and viridiflorene	Target of SARS-CoV-2 (M^{pro}, ACE-2, S-protein, and RdRp as reported in the *in silico* study	Gowrishankar et al. (2021)
Eucalyptus globules	Ellagic acid and apigenin-7-O-glucuronide	Inhabits SARS-CoV-2 M^{pro}	
Justicia adhatoda	Anisotine and vasicolinone	Blocks viral replications	
Theobroma cacao	Amentoflavone, naringin isorhoifolin N3, rutin, and isorhoifolin	Revealed activity to interfere with M^{pro} Isorhoifolin and rutin seem to bind more strongly than N3 co-crystallized inhibitor	Yañez et al. (2021)
Citrus fruits	Hesperidin and hesperetin	Halts the interaction between viral S protein and ACE2 receptor and suppresses the ACE2 and TMPRSS2 expression	Cheng et al. (2021)
Lianhuaqingwen (LH) capsule	Consists of several plants including *Lonicera japonica* and *Forsythia suspense*	*Lonicera japonica* and *Forsythia suspensa* could block the binding of SARS-CoV-2 with ACE2	Hu et al. (2021b)
		LH conferred suppression of the cytopathic effect of SARS-CoV-2 *in vitro* and reduced the viral loads in the cytoplasm and cellular membrane	Runfeng et al. (2020)
Nigella sativa (black seed)	Phenolic compounds, flavonoids, phytosterols, alkaloids, glycosides, and volatile oils	It affects binding at the site of N3 in the SARS-CoV-2 M^{pro}	(Maideen, 2020; Puttaswamy et al., 2020)
Eucalyptus globulus	Apigenin-7-O-glucuronide (AG) and ellagic acid from leaves	*In silico* study reported the inhibitory effects against M^{pro}, ACE-2, S-protein, and RdRp. AG inhibits RdRp complex higher than remdesivir	Gowrishankar et al. (2021)
Justicia adhatoda	Vasicolinone and Anisotine		
Vitex negundo	Eudesmol and viridiflorene		
Murraya koenigii (L.) Spreng	Bismahanine	Binds with the spike protein	Puttaswamy et al. (2020)
Hypericum perforatum L.	Hypericin	Inhibits SARS-CoV-2 M^{pro}	
Cephalotaxus wilsoniana Hayata	Taiwanhomoflavone A	Targets RdRp at 9.8 6 kcal/mol	Joshi et al. (2021)
Ginkgo biloba L	Amentoflavone	SARS-CoV-2 M^{pro}	Puttaswamy et al. (2020)
Vitis vinifera	δ-Viniferin		Joshi et al. (2021)

(Continued on following page)

TABLE 10 | (*Continued*) Herbs using against COVID-19 management.

Herb name/scientific name	Active herb components/whole herbs	Potential mechanism of action against SARS-CoV-2	Reference
		Interacting ability of δ-viniferin with M^{Pro}, RdRp, and hACE-2 suggests its high potential as a multi-target directed ligand against SARS-CoV-2	
Justicia adhatoda	Anisotine	Anisotine potentially inhibits the spike protein and M^{pro}	Kar et al. (2020)
Swertia chirata	Amarogentin	Amarogentin potentially inhibits RdRp	
Psorothamnus arborescens	3,3-Dimethylallyl isofavone	Binds with $3CL^{pro}$	Tahir Ul Qamar et al. (2020)
Myrica cerifera	Myricitrin	Binds with $3CL^{pro}$	
Schisandra sphenanthera	Excavatolide M cembranolide durumolide K	Potential inhibitor of TMPRSS2	Rahman et al. (2020)
Aloe barbadensis	Rhein	Binds with M^{pro}	Narkhede et al. (2020)
Berberis aristata	Berberine	Binds with M^{pro}	
Moringa oleifera	Isorhamnetin, kaempferol, apigenin, rutinoside, and vitexin	Inhibits SARS-CoV-2 M^{pro}	(Mathpal et al., 2021; Sen et al., 2021)
Scutellaria baicalensis Georgi	Baicalin and baicalein	Baicalein showed stronger binding affinity than baicalin to inhibit RdRp	Zandi et al. (2021)
Propolis *Baccharis dracunculifolia*	Limonin, quercetin, and kaempferol	Inhibits RdRp, also inhibit binding with spike protein TMPRSS2 and ACE2	Berretta et al. (2020)
Uncaria tomentosa (Cat's claw)	Proanthocyanidins, epicatechin, proanthocyanidin B2, B4, proanthocyanidin C1, speciophylline, uncarine F, and cadambine	Inhibits M^{pro}, $3CL^{pro}$, interfere ACE2, and spike protein binding	Yepes-Pérez et al. (2020)
Uncaria tomentosa (Cat's claw)	Hydroalcoholic extract of *U. tomentosa* stem bark	Inhibits the release of infectious particles, reducing the cytopathic effect on Vero E6 cells	Yepes-Perez et al. (2021)
Scutellaria barbata D. Don	Scutellarin, baicalein luteolin, naringenin, and wogonin	Effectively inhibits M^{pro} and TMPRSS2 activity *in vitro*	Huang et al. (2021)
Citrus fruits	Hesperidin	More interactive with the SARS-CoV-2 PR protein	Kodchakorn et al. (2020)
Sesamum indicum	Sesamin		
Peanuts (*Arachis hypogea*)	Resveratrol	Significantly decrease the expression of ACE2, modulating host immune response	(Filardo et al., 2020; de Ligt et al., 2021)

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ACE2, angiotensin-converting enzyme-2; 3CLpro, 3 chemotrypsin-like protease; M^{pro}, main protease; RdRp, RNA-dependent RNA, polymerase; TMPRSS2, transmembrane protease serine 2.

anti-cytokine, and antifibrotic properties of curcumin may assist in quick recovery of severe COVID-19 patients (Lelli et al., 2017; Wichmann, 2020). Future clinical studies are warranted to develop standard dosages of curcumin to assess possible clinical benefits in patients with COVID-19 (Ho et al., 2021). After compelling previous therapeutic evidence of *N. sativa* and recent molecular docking findings, a recent review suggests that some bioactive compounds of *N. sativa*, for example, α-hederin, nigellidine, and thymoquinone, could be used as alternative potential herbal drugs to treat COVID-19 (Islam et al., 2021). Another recent review has updated the current status of the naturally occurring compounds such as alkaloids, terpenes, flavonoids, and benzoquinones from different herbs against SARS-CoV-2 infection and suggested that accurate experimental investigation of these compounds may provide insightful information for the potential therapy of COVID-19 patients (Romeo et al., 2021).

13 GENETIC TESTING FOR COVID-19 TREATMENT: PANEL OF GENE CONSIDERATIONS

Plenty of genes of interest that may either be involved in the severity of COVID-19 progression or may potentially modify the PK/PD profiles of COVID-19 therapeutics, and may therefore potentially affect the safety or effectiveness of these medications have been identified in this review. From reviewing the previous information, we enlisted a panel of genes as two categories: 1) mandate genetic test and 2) recommendations for the genetic test.

13.1 Mandate Genetic Test

13.1.1 Mandate Genetic Test for COVID-19 Severity

As described and found evidence in this review, we strongly mandate the genetic test for *HLA*, *ACE2*, and *TMPRSS2* genes for assessing the severity of COVID-19 associated with the genetic variants of these genes.

13.1.2 Mandate Theranostics

Some of the drugs used either as repurposely to combat SARS-CoV-2 infection or used as supportive care for alleviating complications associated with COVID-19 have already well-established evidence for considering pharmacogenomics interventions, and different international pharmacogenomic working groups such as Clinical Pharmacogenetics Implementation Consortium (CPIC) have provided pharmacogenomic-based dosing clinical recommendations as shown in **Table 11**.

At the infancy stage where almost no pharmacogenomics study of COVID-19 therapeutics in this unprecedented health

TABLE 11 | CPIC pharmacogenomic-based dosing guidelines for drug using in COVID-19.

COVID-19 therapeutics (repurposed/supportive care)	Genetic variants	Clinical recommendations	Strength of recommendations	Reference
Atazanavir	*UGT1A1*6*, **28*, **36*, **37*, **80*	NM/IM: Standard therapy PM: Alternative therapy	Strong Strong	Gammal et al. (2016)
Efavirenz	*CYP2B6*4*, **6*, **18*, **22*	UM/RM/NM: Standard dose IM: Start reduced dose (400 mg/day) PM: Start reduced dose (400 mg/day or 200 mg/day)	Strong Moderate Moderate	Desta et al. (2019)
Clopidogrel	*CYP2C19*2*, **3*, **17*	UM/NM: Standard dose IM: Alternative antiplatelet, for example, prasugrel or ticagrelor PM: Alternative antiplatelet, for example, prasugrel or ticagrelor	Strong Moderate Strong	Scott et al. (2013)
Warfarin	*CYP2C9*2*, **3* and *VKORC1* (rs9923231)	Calculate dose for patients carrying these variants based on published validated pharmacogenetic algorithms	Strong	Johnson et al. (2017)
NSAIDs (celecoxib, flurbiprofen, ibuprofen, and lornoxicam)	*CYP2C9*2*, **3*	NM and IM (AS = 1.5): Standard dose IM (AS = 1): Lowest standard dose PM: Reduce 25–50% of the lowest recommended dose	Strong for NM, moderate for IM Moderate Moderate	Theken et al. (2020)
Meloxicam	*CYP2C9*2*, **3*	NM/IM (AS = 1.5): Standard dose IM (AS = 1): Recommends 50% reduction of the lowest standard dose PM: Alternative therapy	Strong for NM, moderate for IM Moderate Moderate	
Piroxicam and tenoxicam	*CYP2C9*2*, **3*	NM/IM (AS = 1.5): Standard dose IM (AS = 1): Alternative therapy PM: Alternative therapy is recommended	Strong for NM, moderate for IM Moderate/optional Moderate/optional	

Here, CPIC, Clinical Pharmacogenetics Implementation Consortium; NM, normal metabolizer; UM, ultrarapid metabolizer; RM, rapid metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; NSAIDs, non-steroidal anti-inflammatory drugs.

situations, we strongly mandate to undertake at least theranostics for these drug–gene pairs (atazanavir–*UGT1A1*, *ABCB1*, *SLCO1B1*, and *APOA5*; efavirenz–*CYP2B6*; nevirapine–*HLA*, *CYP2B6*, and *ABCB1*; lopinavir–*SLCO1B3* and *ABCC2*; ribavirin–*SLC28A2*; tocilizumab–*FCGR3A*; ivermectin–*ABCB1*; oseltamivir–*CES1* and *ABCB1*; clopidogrel–*CYP2C19* and *ABCB1*, warfarin–*CYP2C9* and *VKORC1*; NSAIDs–*CYP2C9*) in patients with COVID-19 based on the evidence of drug–gene interactions for optimizing the safety or efficacy of COVID-19 therapies.

13.2 Recommendations for Theranostics

After evaluating PK properties and low evidence of pharmacogenomic associations, we recommend these drug–gene pairs (remdesivir–*CES1*, *CYP2C8*, *CYP3A4*, and *CYP2D6*; azithromycin–*ABCB1*; losartan–*ABCB1* and *CYP2C9*; lopinavir/ritonavir–*ABCB1*) for further considerations in clinical studies to establish evidence for genetic associations with the safety or effectiveness of COVID-19 medications.

It is reasonable to understand that at the beginning of emergency pandemic situations, clinicians may not be able to prioritize pharmacogenomics intervention issues of COVID-19 drugs; probably that might be one of the best reasons for higher mortality of COVID-19 patients due to not well clinically managed of these patients. Also, although pharmacogenomics is starting to incorporate into routine clinical practice in some parts of the world, for example, United States, Thailand, United Kingdom, and Netherlands, clinicians still are not well-positioned to consider pharmacogenomics recommendations due to either low understanding of this newly evolving area or may not have adequate training regarding the pharmacogenomic uptake in the clinical practice. However, COVID-19 situations are stabilizing slowly, and this is the high time for clinicians/genomics researchers to investigate pharmacogenomics associations of drugs in this clinical condition.

Since no pharmacogenomic study assessing the associations of COVID-19 therapeutics with the safety or efficacy has not been either undertaken or published yet, we suggest to consider a panel of genes of interest which have already been discussed above in this review to assess the impacts of pharmacogenomics in COVID-19 therapeutics to establish precision medicine of COVID-19, as illustrated in **Figure 6**.

14 PHARMACOGENOMICS AND PRECISION MEDICINE FOR COVID-19 IN THAILAND

Thai population might be at particular risk for either developing severe COVID-19 due to the *HLA* genetics or developing toxicities/therapeutic ineffectiveness by COVID-19 drugs. This is partly because Thai population has great diversity of *HLA*, transporters, and *CYP* genetic variants. It has been previously reported that ~25% of the Thai population carried *HLA-DQA1*/*HLA-DQB1* genetic polymorphisms (Puangpetch et al., 2014;

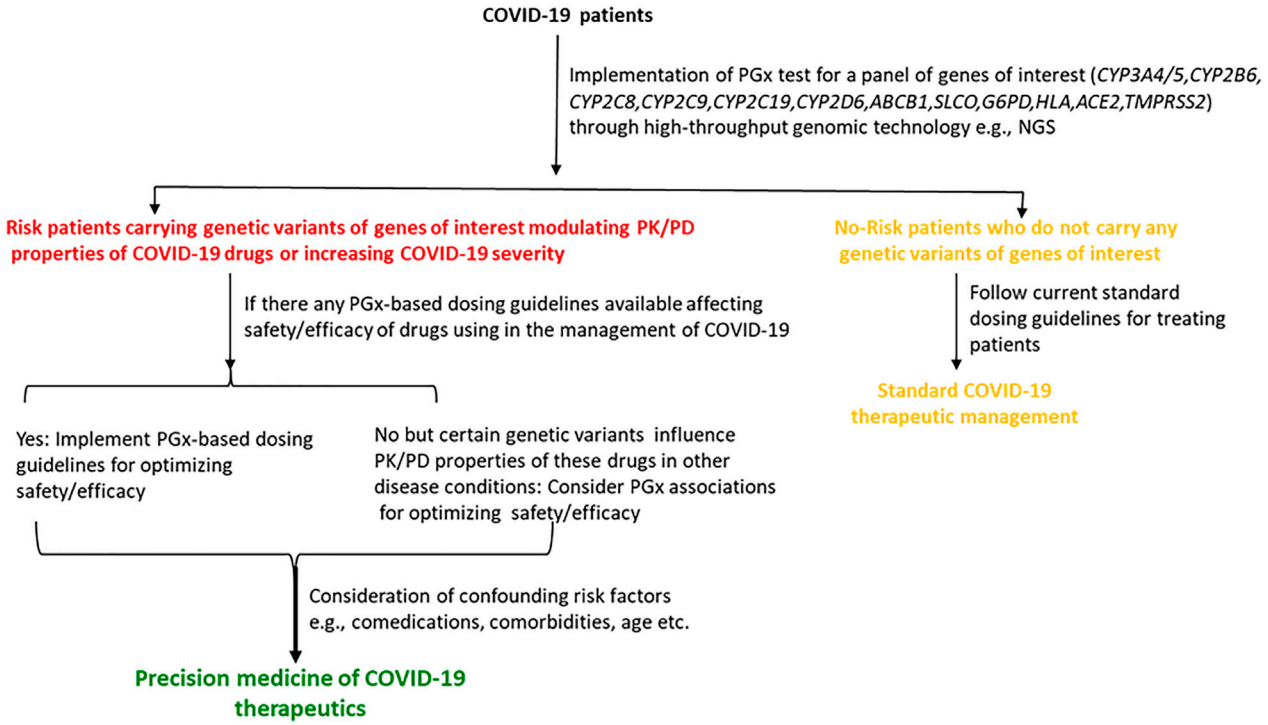

FIGURE 6 | Implementation of precision medicine for drugs used in the management of COVID-19. Here, COVID-19, coronavirus disease-2019; NGS, next-generation sequencing; PK, pharmacokinetics; PD, pharmacodynamics.

Satapornpong et al., 2020), which might render COVID-19 severity. Minor allele frequencies of the *CYP2C9*2* and *CYP2C9*3* in Thai population were 0.08 and 5.3%, respectively. Minor allele frequencies of the *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* in Thai population were 25.6, 2.5, and 1.8%, respectively. Approximately 30% of the *CYP3A4* variant allele was identified in the Thai population as reported elsewhere (Sukprasong et al., 2021). Over 30% Thai population carried *C3435T ABCB1* genetic polymorphisms as revealed in a previous study conducted in Thailand (Sensorn et al., 2013). Overall, it is concluded that considerable proportion of Thai population might be at risk of either severe COVID-19 manifestation or might be at risk of developing toxicities/ineffectiveness of the COVID-19 medications due to carrying these genetic variants. Moreover, the herbs especially andrographolide and the others as described in this review are commonly used in this population; this might also render the risk of toxicities/ineffectiveness of these herbs due to either DHIs or herbogenomics.

15 CLINICAL PERSPECTIVE

To our best knowledge, no clinical studies were identified in the literature to date that had assessed either metabolic or transporter genetic variants with the safety or effectiveness of current COVID-19 therapeutics. This is creating evidence impasse and delaying the target for finding appropriate therapeutics to combat COVID-19 successfully. From considering the PK/PD profiles of the current COVID-19 therapeutics under investigation as discussed in this review, it is emerging the needs for assessing genetic associations of the relevant metabolic or transporter genes of interest for optimizing the safety or effectiveness of COVID-19 therapeutics. Future clinical studies or trials are warranted to investigate such genetic associations for the achievement of precision medicine for COVID-19. Since it is well evidenced that the mortality is significantly higher in older people and having comorbidities (Biswas et al., 2021a), DDIs should also be considered in these assessments because of vulnerability to polypharmacy. Ideally, considerations of multifactorial drug–gene interactions (DGIs) of COVID-19 therapeutics may accelerate the development of precision medicine of COVID-19 in the real clinical settings as shown in **Figure 7**, as established in other therapeutic areas such as antiplatelets (Biswas et al., 2021b).

It is very important to note here that this predictive model has considered only a pharmacologically active drug involving CYP metabolism; however, in case of a prodrug, the effects will be *vice versa*, and this model is also applicable to other genes, for example, transporter genes affecting the safety or efficacy of COVID-19 drugs.

16 CONCLUSION

The global outbreak of SARS-CoV-2 has evolved into an emergent COVID-19 pandemic causing huge morbidity and

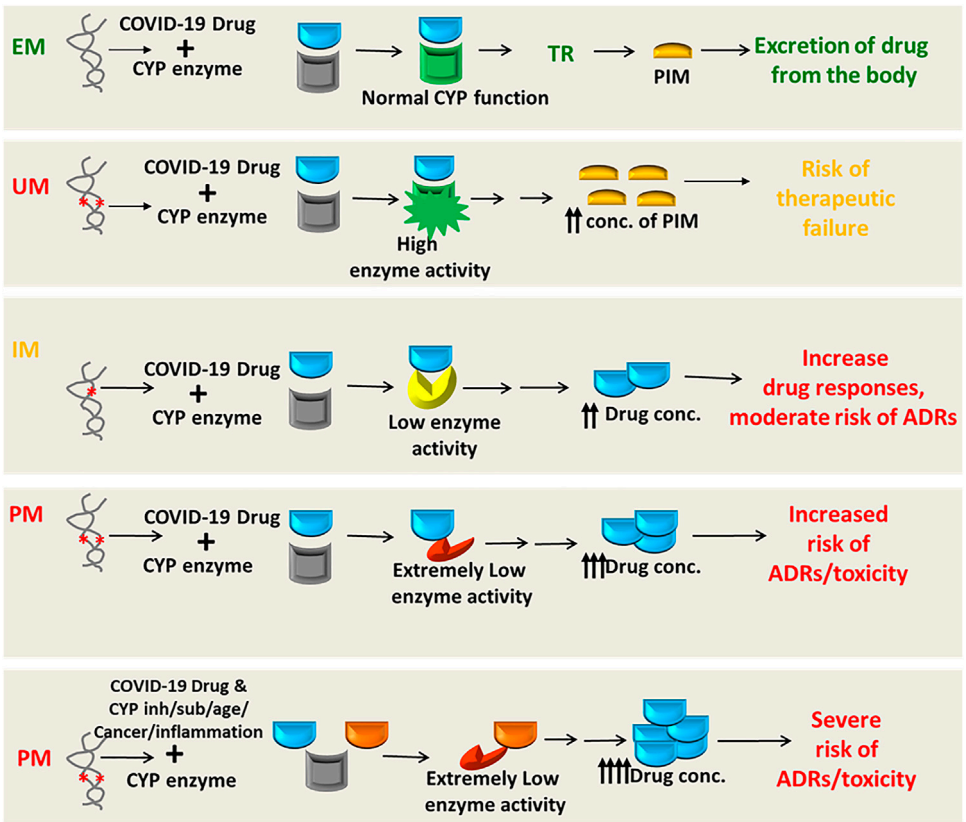

FIGURE 7 | Predictive model of multifactorial DGIs for a COVID-19 drug showing possible effects of *CYP* gene variants representing likely phenotypes associated with the risk of therapeutic failure or ADRs/toxicity. Here, DGIs, drug–gene interactions; COVID-19, coronavirus disease-2019; EM, extensive metabolizer; UM, ultrarapid metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; CYP, cytochrome P450 enzyme; *indicates *CYP* gene variant; Inh, inhibitor; Subs, substrate; TR, therapeutic response; PIM, pharmacologically inactive metabolite; ADRs, adverse drug reactions; conc., concentration.

mortality in the world. Many drugs without establishing clinical effectiveness or tailoring safety are being administered to tackle COVID-19 pandemic situations. A repurposing strategy might be more effective and successful if pharmacogenetic interventions of these drugs are being considered in future clinical studies/trials. Safety and effectiveness of several repurposed drugs currently being used for the management of COVID-19 may be affected by the *CYP*/transporter genetic variants. From reviewing the current evidence of pharmacogenetic of these drugs in either COVID-19 or other diseases, we strongly mandate to undertake pharmacogenetic assessment for at least these drug–gene pairs (atazanavir–*UGT1A1*, *ABCB1*, *SLCO1B1*, and *APOA5*; efavirenz–*CYP2B6*; nevirapine–*HLA*, *CYP2B6*, and *ABCB1*; lopinavir–*SLCO1B3* and *ABCC2*; ribavirin–*SLC28A2*; tocilizumab–*FCGR3A*; ivermectin–*ABCB1*; oseltamivir–*CES1* and *ABCB1*; clopidogrel–*CYP2C19* and *ABCB1*, warfarin–*CYP2C9* and *VKORC1*; NSAIDs–*CYP2C9*) in patients with COVID-19 for advancing precision COVID-19 therapeutics by optimizing the safety or effectiveness of these drugs.

Although it is very unlikely that there are almost no pharmacogenetic data for COVID-19 drugs, from inferring the PK/PD properties and some pharmacogenetic evidence of these drugs in other diseases/clinical conditions, it is highly likely that pharmacogenetic associations are also feasible in at least some COVID-19 drugs currently being administered as shown in this review and should be considered in future clinical studies/trials. Molecular docking and computational studies are promising to achieve new COVID-19 therapies as shown in this review. Current situation in the discovery of anti-SARS-CoV-2 agents at four important targets from *in silico* studies has been described and summarized in this review. Although naturally occurring compounds from different herbs against SARS-CoV-2 infection are favorable, accurate experimental investigation of these compounds is warranted to provide insightsful information. Moreover, clinical considerations of DDIs and DHIs of the existing repurposed drugs along with pharmacogenetic (e.g., efavirenz and *CYP2B6*) and herbogenetic (e.g., andrographolide and *CYP2C9*) interventions, collectively called multifactorial drug-gene interactions (DGIs), may further accelerate the development of precision COVID-19 therapies in the real-world clinical settings.

AUTHOR CONTRIBUTIONS

CS contributed to the conception, and designed and reviewed the manuscript. MB, NS, and TR contributed to the writing of the

manuscript. NS, KS, MB, and ME drew the figures and summarized the tables. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by grants from the 1) Mahidol University International Postdoctoral Fellowship, Mahidol University; 2) Faculty of Medicine, Ramathibodi Hospital, Mahidol University; 3) the Health System Research Institute under Genomics Thailand Strategic Fund; and 4) the International Research Network-the Thailand Research Fund (No. IRN60W003).

ACKNOWLEDGMENTS

The authors thank the staff of Pharmacogenomic and Personalized Medicine of Ramathibodi Hospital.

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 19 September 2023
DOI 10.3389/fphar.2023.1268119

OPEN ACCESS

EDITED BY
Francesca Coperchini,
University of Pavia, Italy.

REVIEWED BY
Federica Galimberti,
IRCCS MultiMedica Hospital Sesto San Giovanni, Italy
Gustavo B.F. Oliveira,
Dante Pazzanese Institute of Cardiology (IDPC), Brazil
Martin Duerden,
Freelance, United Kingdom

*CORRESPONDENCE
Akila Prashant,
akilaprashant@jssuni.edu.in

†These authors have contributed equally to this work

RECEIVED 27 July 2023
ACCEPTED 08 September 2023
PUBLISHED 19 September 2023

CITATION
Birla S, Angural A, Madathumchalil A, Shende RV, Shastry SV, Mahadevappa M, Shambhu SK, Vishwanath P and Prashant A (2023), Redefining the polypill: pros and cons in cardiovascular precision medicine.
Front. Pharmacol. 14:1268119.
doi: 10.3389/fphar.2023.1268119

Redefining the polypill: pros and cons in cardiovascular precision medicine

Siddharth Birla[1], Arshia Angural[2], Arya Madathumchalil[2†], Ritika V. Shende[2†], Sharvani V. Shastry[2†], Manjappa Mahadevappa[3], Sunil Kumar Shambhu[3], Prashant Vishwanath[1] and Akila Prashant[1,2]*

[1]Department of Biochemistry, JSS Medical College, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagara, Mysuru, Karnataka, India, [2]Department of Medical Genetics, JSS Medical College, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagara, Mysuru, Karnataka, India, [3]Department of Cardiology, JSS Medical College and Hospital, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagara, Mysuru, Karnataka, India

Polypill is a multi-drug formulation in a single pill intended to simplify the drug regimen and reduce medication-induced adverse effects. The most common multidrug combinations in a polypill are used to treat cardiovascular diseases and are targeted against key modifiable risk factors such as hypertension and hyperlipidemia. These contain blood-pressure lowering agents, statins, and anti-platelet agents usually in a fixed dose. Polypills can be an affordable therapeutic intervention for treating high-risk patients, as these are proven to increase patients' adherence to medication and improve clinical outcomes. Over the previous years, randomized clinical trials of several polypills have yielded contradictory findings, raising skepticism regarding their widespread use in primary disease prevention. Here, we have reviewed the concept of polypills, the evidence-based strengths, the limitations of this polypharmacy intervention strategy, and discussed future directions for their use in the primary and secondary preventive management of cardiovascular diseases and associated risk factors.

KEYWORDS

polypill, LDL, hypertension, CVD, NAFLD, multi-drug, precision medicine, genomics

1 Introduction

Cardiovascular diseases (CVDs) are the leading cause of disability and premature death, contributing to the highest morbidity and mortality rates globally, especially in low- and middle-income countries (LMICs). Moreover, the CVD-associated morbidity rate has been projected to enhance, surpassing that of communicable diseases (Laslett et al., 2012). Despite

Abbreviations: ADRs, Adverse Drug Reactions; ACE, Angiotensin-Converting Enzyme; ALT, Alanine Transaminase; BP, Blood Pressure; CDC, Centers for Disease Control; CV, Cardiovascular; CVD, Cardiovascular Disease; CVE, Cardiovascular Event; CVP, Cardiovascular Polypill; CNIC, Centro Nacional de Investigaciones Cardiovasculares; FDC, Fixed-Dose Combination; FOCUS, Fixed Dose Combination Drug for Secondary Cardiovascular Prevention; HOPE-3, Heart Outcome Prevention Evaluation-3; IHD, Ischemic Heart Disease; LDL-C, Low-Density Lipoprotein Cholesterol; LMICs, Low and Middle Income Countries; MACE, Major Adverse Cardiovascular Events; NAFLD, Non-alcoholic Fatty Liver Disease; pNASH, Presumed Non-Alcoholic Steatohepatitis; RCT, Randomized Clinical Trial; TIPS-3, The International Polycap Study 3; WHO, World Health Organization.

the availability of different cardiovascular (CV) medicines, a significant gap exists in the therapeutic management of CVDs and the prevention of associated risk factors. The most critical challenge faced in the therapeutic management of CVD patients is their poor compliance with the recommended therapy and lifestyle changes. This is mainly attributed to patient's confusion about the consumption of complex therapeutic regimens consisting of a large number of recommended medicines or their antipathy towards these pills, and the affordability of the prescribed medication (Castellano et al., 2014). Furthermore, only some patients are able to receive a full combination of the recommended medicines (Lawlor et al., 2003). Some of the CVD patients may develop recurrent CV event (CVE) within a few years of their first CV episode despite undergoing preventive pharmacotherapy. All of these factors have been linked to poor clinical outcomes in the patients (Perel et al., 2015). However, the occurrence of these can be reduced by the use of preventive fixed-dose combinations (FDCs) of CV drugs, called Polypill, and multiple target medicines which may not increase pill burden among patients. Polypill has been defined as the FDC of several drugs that can control modifiable CVD risk factors or associated disease pathologies (Fuster et al., 2017).

The concept of developing combination therapy formulations or cardiovascular polypill (CVP) as a preventive therapeutic approach was originally proposed in 2001 by the World Health Organization (WHO) and Welcome Trust Expert Group (World Health Organization, 2002). The expert members suggested once-a-day oral administration of a single combination pill containing four drugs including a β-blocker, an angiotensin-converting enzyme inhibitor (ACEI), a statin, and aspirin, each well-documented for their preventive roles in CVD. Later, Yusuf *et al.* reported that there could be a 75% reduction in CVEs upon implementation of a combination of four drugs (aspirin, beta-blocker, statin, and angiotensin-converting enzyme (ACE) inhibitor) in CVD patients (Yusuf, 2002). They had put forward a precept that the first heart attack or stroke event could be prevented by using cumulative effects of various drugs in combination as a single pill or polypill. An FDC of antihypertensive agents, aspirin, lipid-lowering drugs, and sometimes folic acid has been suggested for the wellbeing of patients with a high CVD risk. Nicholas Wald and Malcolm Law suggested six different drug components in a polypill, originally including aspirin, ACE inhibitors, beta-blockers, diuretics, folic acid, and statin. An analysis by Wald and Law demonstrated that there is a drop in myocardial infarction and stroke rates by over 80% when their model of polypill was assessed in individuals aged above 50 years (Wald and Law, 2003). This polypill model could improve platelet function and regulate blood pressure (BP), low-density lipoprotein cholesterol (LDL-C), and serum homocysteine levels within the optimum biological range in CVD-risk patients (Table 1). Purportedly, this formulation can reduce the risk of ischemic heart disease (IHD) and stroke by 46% and 63%, respectively, whereas statins can lower the mean LDL-C levels by 1.8 $mmolL^{-1}$, decreasing IHD risk and stroke by 60% and 17%, respectively (Law et al., 2003b). The CVPs were, thereby, recommended by them to improve the key CVD risk factors.

A fixed half-dose formulation of multiple antihypertensive drugs is reported by several studies to lower the elevated BP and other associated risk factors with relatively less adverse effects in comparison to a full dose. Chief health organizations like the Centers for Disease Control (CDC), the WHO, and the Wellcome Trust have initiated research programs emphasizing the development of polypills and the evaluation of their effects (Combination Pharmacotherapy and Public Health Research Working Group, 2005; Lonn et al., 2010). The European Commission has recently concluded lack of patient adherence to medication as one of the key contributory factors in persistent CVEs, and strongly advocated the use of polypills to address this problem (Fuster et al., 2017). Subsequently, CVPs are being scrutinized as an alternative therapy for improving therapeutic outcomes for CVD patients. In 2018, the European Society of Cardiology provided clinical guidelines on making CVP as an integral part of the comprehensive secondary prevention of CVD (Ibanez et al., 2017). The 2018 European Society of Hypertension and the 2018 and 2020 updates of the American Heart Association Hypertension guidelines have further endorsed the use of CVPs in the CVD management (Whelton et al., 2018; Williams et al., 2018;

TABLE 1 The table reveals the outcomes of Wald and Law's cardiovascular polypills on ischemic heart disease and stroke after 2 years of treatment (Wiley and Fuster, 2014).

Risk factor	Drug agents	Reduction in risk factor	
		IHD (in %); 95% CI	Stroke (in %); 95% CI
Blood pressure	Three different classes of antihypertensive[#] drugs at a half-standard dose	46; 39–53	63; 55–70
LDL-C	Statin*	61; 51–71	17; 9–25
Serum homocysteine	Folic acid (0.8 mg/day)	16; 11–20	24; 15–33
Thrombosis	Aspirin (75 mg/day)	32; 23–40	16; 7–25
Combined effect		88; 84–91	80; 71–87
Combined effect (omitting folic acid)		86	74
[#]Thiazides, beta-blockers, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, and calcium channel blockers			
*Atorvastatin (10 mg), Simvastatin, or Lovostatin (80 mg taken in the morning or 40 mg taken in the evening)			
Abbreviations: LDL-C = Low Density Lipoprotein Cholesterol; IHD = Ischemic Heart Disease; CI = Confidence Interval			

TABLE 2 A list of some cardiovascular polypills that have undergone clinical trials previously and the potential inter-compositional interactions.

Brand name	Manufacturer	Constituents	Potential compositional (inter-drug) Interactions*
Polycap™	Cadila Pharmaceuticals Ltd., India	aspirin (100 mg), atenolol (50 mg), hydrochlorothiazide (12.5 mg), ramipril (5 mg), simvastatin (20 mg)	Aspirin may decrease the antihypertensive activities of Atenolol
			Aspirin may decrease hydrochlorothiazide's excretion rate, which could result in a higher serum level
			The metabolism of aspirin can be decreased when combined with Simvastatin
			The therapeutic efficacy of Atenolol can be increased when combined with hydrochlorothiazide
			Atenolol may decrease Simvastatin's excretion rate, which could result in a higher serum level
			Ramipril may increase the hypotensive activities of Atenolol
Polypill	Cipla, India	amlodipine (2.5 mg), hydrochlorothiazide (12.5 mg), losartan (25 mg), simvastatin (40 mg)	The serum concentration of Simvastatin can be increased when combined with amlodipine
			Simvastatin may decrease losartan's excretion rate, which could result in a higher serum level
PolyIran	Alborz Darou Pharmaceutical Company, Iran	aspirin (81 mg), enalapril (5 mg); or	The therapeutic efficacy of Enalapril can be decreased when combined with aspirin
		atorvastatin (20 mg), hydrochlorothiazide (12.5 mg), valsartan (40 mg)	The excretion of atorvastatin can be decreased when combined with Valsartan
Ramitorva®	Zydus Cadila, India	aspirin (75 mg), ramipril (5 mg), atorvastatin (10 mg)	no compositional inter-drug interactions
Red Heart Pill™ 1	Dr Reddy's Laboratories, India	atenolol (50 mg), aspirin (75 mg), lisinopril (10 mg), simvastatin (40 mg)	Aspirin may decrease the antihypertensive activities of Atenolol
			The metabolism of aspirin can be decreased when combined with Simvastatin
			The therapeutic efficacy of Lisinopril can be decreased when combined with aspirin
			Atenolol may increase Lisinopril's hypotensive activities and decrease Simvastatin's excretion rate, which could result in a higher serum level
Red Heart Pill™ 2	Dr Reddy's Laboratories, India	aspirin (75 mg), hydrochlorothiazide (12.5 mg), lisinopril (10 mg), simvastatin (40 mg)	Aspirin may decrease hydrochlorothiazide's excretion rate, which could result in a higher serum level
			The therapeutic efficacy of Lisinopril can be decreased when combined with aspirin
			The metabolism of Aspirin acid can be decreased when combined with Simvastatin
Starpill®	Cipla, India	aspirin (75 mg), atenolol (50 mg), atorvastatin (10 mg), losartan potassium (50 mg)	Aspirin may decrease the antihypertensive activities of Atenolol
			The risk or severity of renal failure, hyperkalemia, and hypertension can be increased when losartan is combined with aspirin
			Atenolol may decrease the excretion rate of atorvastatin, resulting in a higher serum level
			Atenolol may increase the arrhythmogenic activities of losartan
			The metabolism of atorvastatin can be decreased when combined with losartan

(Continued on following page)

TABLE 2 (*Continued*) A list of some cardiovascular polypills that have undergone clinical trials previously and the potential inter-compositional interactions.

Brand name	Manufacturer	Constituents	Potential compositional (inter-drug) Interactions*
Trinomia®/ Sincronium®	Ferrer Internacional, Spain/Hexal, Germany	aspirin (100 mg), atorvastatin (20 mg), ramipril (2.5, 5 or 10 mg)	no compositional inter-drug interactions
Trinomia®	Ferrer Internacional, Spain	aspirin (100 mg), ramipril (2.5, 5 or 10 mg), simvastatin (40 mg)	The metabolism of aspirin can be decreased when combined with Simvastatin
Zycad™	Zydus Cadila, India	aspirin (75 mg), atorvastatin (10 mg), metoprolol (50 mg), ramipril (5 mg)	Aspirin may decrease the antihypertensive activities of Metoprolol
			Ramipril may increase the hypotensive activities of Metoprolol
*Information is retrieved from DrugBank Online tool (URL: https://go.drugbank.com/)			

Williams et al., 2020). The first CVP was developed by Centro Nacional de Investigaciones Cardiovasculares (CNIC)—Ferrer, Europe, which recently became accessible as a general prescription for ameliorating CVD (Fuster et al., 2017).

Being an alternative to the already existing complex multi-drug regimen, combining or scaling up a package of individual anti-CVD drugs into a polypill has several benefits. The cost-effectiveness and simpler usage of polypills improve patient compliance with the treatment since these are widely accepted by the patients (Gnanenthiran et al., 2023). As these do not require any dose adjustment, the FDC in polypill makes it safer to use for controlling BP (Muñoz et al., 2019).

2 The combination therapy: polypill

A polypill is usually defined as an FDC of several pharmaceutical components in variable compositions that have demonstrated benefits against CVD and the associated modifiable secondary complications without significant adverse effects (Wald and Law, 2003). Many studies have reported the use of polypills in both primary as well as secondary prevention of CVD risk factors. These usually contain aspirin (50–125 mg), a potent statin (10 mg atorvastatin or 40–80 mg simvastatin), folic acid (0.8 mg), and three blood pressure-lowering drugs (beta blockers, angiotensin receptor blockers, ACE inhibitors, calcium channel blockers, and thiazide diuretics) at half the standard dose (Wald and Law, 2003; Lonn et al., 2010). Furthermore, polypills can be either multi- or single-purpose based on their scope of action against CVD-associated risk factors. A single FDC can be either targeted against each major CVD risk factor (known as a multi-purpose polypill) or can control only one risk factor (known as a single-purpose polypill) (Sukonthasarn et al., 2021).

Polypills can prevent CVD in a population-wide setting, owing to their potential advantages over conventional therapies, as mentioned elsewhere (Law et al., 2003a). The usage of a polypill, such as a gelatin capsule securing losartan (25 mg), atorvastatin (10 mg), hydrochlorothiazide (12.5 mg), and amlodipine (2.5 mg), has been recently demonstrated to cause a reduction in LDL-C levels and systolic BP (Muñoz et al., 2019). In the past few years, several polypills with different compositions, at least containing one antihypertensive, lipid lowering drug with or without aspirin, have been developed evaluated in different clinical trials, registered and marketed for secondary prevention of CVDs (Patel et al., 2022). A list of some of these CVPs is provided in Table 2.

3 Issues to be addressed before implementing the polypill

Before the therapeutic implementation of the polypill, there must be a substantiation that it would cut down the occurrence of major CV illnesses and be safe enough for use as primary prevention in middle-aged individuals. Ideally, the multicombination drug has four to five active drug components in the form of an FDC. Therefore, the pharmacokinetics, bioavailability, risk factors, and adverse drug reactions (ADRs) as well as potential inter-drug interactions need to be documented for individual compositions of the polypill formulation, even though the effects of the polypill's components, when combined, are seen to be cumulative (Patel et al., 2010). However, using Polypill does not contradict the effect of individualized treatment, rather it is considered as an appended therapy for persistent elevation in BP or LDL-C levels (Lonn et al., 2010). Certain factors are vital to the successful implementation of any FDC, including the cost- and dose-effectiveness of drugs used to formulate the polypills and their safe implementation based on the physicochemical compatibility within the individual components of the pill. Other considerable factors include effortless treatment methods, dosing time, intake of pills, and their respective side effects (Smith, 2009). The potency and safety of these drugs can be determined through clinical trials focussed on understanding the cumulative effects of the FDCs. Most widely acknowledged national and international therapeutic guidelines are now focussing on BP lowering and LDL-C regulating therapeutic objectives as a result of confirmed positive results from several clinical trials on drugs lowering the BP- and LDL-C levels (Sukonthasarn et al., 2021).

The CVPs have practically remained underutilized in clinical settings across the globe. This has been attributed to different issues including the physician's personal perspectives on CVPs due to lack of established evidence base, their practical inexperience in using combination therapies and inability to titrate individual drug in order to achieve desired therapeutic outcome, some patient factors and barriers to health system (Webster et al., 2020; Patel et al., 2022; Gnanenthiran et al., 2023; Khan et al., 2023).

4 The pros and cons of polypill

Since the first description of the polypill concept, several clinical trials with different CVPs have demonstrated improved medicine adherence among patients and therapeutic outcomes compared to conventional therapies. Polypills have been reported as an effective alternative and preventive (primary and secondary) therapeutic measure by many researchers.

Initially, the international Heart Outcome Prevention Evaluation-3 (HOPE-3) study conducted in three phases evaluated the role of three drugs in over 1,200 participants with at least one CV risk factor, including men over 55 years and women over 65 years. The study did not report any significant reduction in the primary outcome of CV mortality, non-fatal myocardial infarction or stroke in patients receiving only BP-lowering therapy (a low dose of a candesartan/thiazide diuretic hydrochlorothiazide) compared to those receiving placebo (Lonn et al., 2016). Whereas, the participants who received only the statin (rosuvastatin 10 mg) showed a relative lower risk of CVEs by 24% compared to those receiving placebo (Yusuf et al., 2016a). The participants who received the combination of two hypertensive drugs and the statin displayed significantly lower rates of the primary outcome compared with those receiving double placebo (Yusuf et al., 2016b).

The PolyIran study had previously indicated that the use of polypill significantly helps in the primary prevention of CVD, reducing the risk of major CV events in individuals with or without any previous CVD history (primary prevention) by 20% and 40%, respectively (Roshandel et al., 2019). Although this analysis did not show any significant interaction, the results might suggest that the use of polypills could be considered for primary prevention. Findings of a randomized clinical trial (RCT), named The International Polycap Study 3 (TIPS-3), revealed that participants with intermediate CV risks but without CVD, who received combined treatment with polypill and aspirin had better CV outcomes than placebo (Yusuf et al., 2020). A recent international, multi-centric, randomized phase-III clinical trial of the CNIC-Polypill, named the VULCANO study, conducted for 16 weeks in 499 subjects (at high or very high risk without a previous CVE) from 47 centres assessed the impact of this multi-drug formulation on LDL-C and systolic BP levels (Mostaza et al., 2022). This study reported the CNIC-Polypill as "safe-to-use" and can be a suitable multi-drug therapeutic strategy in preventing CVD and controlling associated risk factors.

Furthermore, the findings of the FOCUS (Fixed Dose Combination Drug for Secondary Cardiovascular Prevention) project have highlighted the role of FDC in the secondary prevention of CV events (Castellano et al., 2014). A recent observational retrospective study, the NEPTUNO study, assessed the effectiveness of the CNIC-Polypill in secondary prevention. The study findings suggested a significant reduction in the incidence of recurrent major adverse CVEs (MACE), total cholesterol and systolic BP in a group of patients with established atherosclerotic CVD and who were on the polypill treatment (test group) when compared with those on monotherapy (González-Juanatey et al., 2022). Other therapeutics outcomes such as improved blood pressure and medicine adherence in the test group were better compared to the study control groups. In a yet another RCT study named the SECURE (Secondary Prevention of Cardiovascular Disease in the Elderly) trial, efficacy of a polypill containing aspirin (100 mg), ramipril (2.5, 5, or 10 mg) and atorvastatin (20 or 40 mg) was assessed with respect to medication adherence and major CV outcomes in 2,499 patients aged over 65 years who had Type I myocardial infarction (Castellano et al., 2022). The authors suggested a significant risk reduction in MACE among those recruited subjects, who had a relatively higher rate of adherence to the trialled polypill.

Recently, a number of meta-analyses on these RCTs suggested the overall effectiveness or outcomes of polypill therapy in medicine persistence, prevention and therapeutics of CVDs. One such study by Rao et al. (2022) evaluated polypill's influence on the CVEs and associated risk factors (Rao et al., 2022). This study supported the fact that the usage of CVPs significantly improve adherence to medication without any association between their use and rates of adverse events or drug discontinuation. It was further reported that their use results in risk reduction for MACE among low-risk and primary prevention patients, overall reduction in CVD risk factors and the risk of all-cause mortality. Another meta-analysis of RCTs by Mohamed et al. (2022) reported a positive association between the use of polypills and reduction in levels of clinical risk factors including BP (both diastolic and systolic), total cholesterol, LDL-C, CVD mortality and MACEs (Mohamed et al., 2022). Furthermore, Hennawi et al.'s (2023) meta-analysis of 18 RCTs with a total participation of 20,463 individuals evaluated the efficacy of polypill therapeutic approach in reducing CV risk factors such as hypertension and dyslipidemia (Hennawi et al., 2023). This study reported a statistically significant association between polypill therapy and improved medicine adherence as well as reduction in BP (both systolic and diastolic) and total cholesterol levels in high-risk individuals or those with confirmed CVD diagnosis. Initially, the concept of using a polypill in CVD management led to conjectures (Smith, 2009).

Although there are several benefits to the intake of polypills, it has also been proven detrimental in many aspects (Webster et al., 2020). A few studies have also pointed to relative contraindications to polypills. Recently published findings of the TIPS-3 clinical trial failed to provide any significant evidence of the polypill's positive impact on improving cognitive and functional decline in people aged over 65 with CV risk factors (Bosch et al., 2023). This polypill, with or without aspirin, was associated with reduced functional decline in these patients without causing any detrimental effects on cognition. The MACEs, such as cardiac death, myocardial infarction, stroke, acute coronary syndrome, revascularisation procedures, development or worsening of heart failure, and development of persistent new arterial fibrillation estimated for 34 months, are some of the other primary outcomes of polypill's randomized clinical trials (Sadeghi et al., 2022). Furthermore, previous clinical trials on CV polypills were conducted on populations from LMICs and the underprivileged population in high-income countries (Muñoz et al., 2019). Though these clinical trials had provided encouraging results in enhancing patient-medication adherence rates, alleviation of CVD risk factors, and fewer adverse events, most of these studies (except the PolyIran Trial) lacked enough statistical power for evaluating the impact of polypill on the clinical outcomes of CVD patients.

Having two sides to the same coin, polypills are not only proven beneficial but there are certain limitations to their use. These include difficulties with dose adjustment to focus on target patients, concern about the consumption of unnecessary medication, and management of too low BP or LDL-C levels. Titration adjustment of individual components within the polypill can be another major challenge while adjusting dose for co-morbid patients, elderly patients, and patients already taking multiple medications. In addition to contradictory evidence for the role of CVPs in improving the clinical outcomes of CVD patients, there are further concerns about their unknown potential adverse consequences (such as bleeding events, and dizziness) in clinically healthy populations taking these pills as a preventive measure. The aforementioned factors can impact the clinical application of polypills for managing BP and other conditions, as these may preclude the recommendation of CVPs by clinicians, subsequently making the patients opt for risk-based therapies with lower treatment thresholds.

Further, there are several additional challenges to the application of polypill, some of which include the technical issues faced while developing an FDC, regulatory hurdles for poorly defined combinations of three or more components, lack of research and development budgets on FDC formulations, and their clinical trials, the unwillingness of a few companies to invest because of the low-profit gains, the ambiguities connected with intellectual property, and other regulatory and fabrication issues (Rafter and Woodward, 2005). Moreover, Walde and Law's statement about taking the polypill by those over 55 years is way too generalized and remained controversial (Wald and Law, 2003). It is not justified whether the polypill is intended for general public use or only for specific populations with a relatively high burden of CVD. It is further questionable whether the individual benefits of each drug component of the polypill are cumulative in the affected populations, and whether a one-sized FDC is effective and safe for everyone without any adverse effects. Although the individual adverse reactions of each component in a polypill are well known (as highlighted in Table 2), it is a challenge to decipher which drug component might have caused an adverse reaction in a suspected drug-related adverse event. Previously conducted clinical trials on one or other polypills assessing the role of adding an extra class of drug component have revealed that these do confer a risk reduction. But none of these trials were based on studying the exact composition like the polypill proposed by Walde and Law. Despite all these, a genre of mini polypills is available (Rafter and Woodward, 2005).

5 Polypill usage in hypertension and NAFLD patients

It has been noted that patients with hypertension and non-alcoholic fatty liver disease (NAFLD) are more vulnerable to major CVDs. Hypertension is a major global health issue and a leading preventable risk factor for premature death and disability worldwide. The swift switch from monotherapy to combination medication is part of the strategy to improve the management of BP. For achieving comparable management of hypertension, multi-drug treatments in a low therapeutic dose have been documented to be more well-tolerated than the corresponding monotherapies in a higher dose (Law et al., 2003a; Wald et al., 2009; Mancia et al., 2014). Furthermore, several recent clinical guidelines have advised multimodal therapy with a combination of two BP-lowering drugs taken in the form of a single pill as the first therapy for the majority of hypertensive patients (Gupta et al., 2010; Sherrill et al., 2011; Mancia et al., 2014; Weber et al., 2014; Lafeber et al., 2016; Brainin et al., 2022). At a broader outset, the polypill approach has been shown to prolong treatment adherence relative to usual care in all CVD patients, while also suggesting a further reduction in concomitant risk factors (Cimmaruta et al., 2018).

NAFLD is the most typical chronic liver ailment prevalent in developed countries. It is assessed that NAFLD will be the leading and one of the foremost causes of liver transplantation by the year 2030 (Tana et al., 2019). Except for the recognized liver-associated morbidity and mortality, a large body of evidence shows that CVD risk represents the foremost cause of death in NAFLD patients (Tana et al., 2019). Recently a 5-year extended clinical trial, named PolyIran-Liver Trial, conducted in a randomized population setting in Iran, investigated the effectiveness of a polypill (aspirin, atorvastatin, hydrochlorothiazide, and valsartan) for the deterrence of MACEs among individuals with and without presumed non-alcoholic steatohepatitis (pNASH). The primary findings of this trial suggested that NAFLD patients consenting to receive the FDC medication had a better outcome against MACE, indicating polypill to be safe and effective against fatty liver disease and enhanced liver enzyme levels (Merat et al., 2022). The use of this multi-drug formulation indeed benefitted NAFLD subjects without pNASH by alleviating the levels of liver enzymes, whereas no changes were observed for those with pNASH after a 60-month follow-up. On the contrary, its administration in participants either with or without pNASH could not indicate any statistical significance in lowering the risk of MACE. The results further indicated that polypill had significantly reduced Alanine Transaminase (ALT) levels in individuals with NAFLD-pNASH.

6 FDC in the welfare of underprivileged, vulnerable groups

A growing number of CVD incidences in LMICs calls for simple and cost-effective therapeutic approaches for primary as well as secondary prevention of CVDs. A polypill-based therapeutic strategy can aid all the communities, especially the most vulnerable and underprivileged ones with fewer resources and facing a relatively higher burden of CVDs and CVD-related mortality. Therefore, these communities must be given primary access to polypills.

It has been proposed by Lim et al. (2007) that almost a fifth of all deaths from CVD could be averted by scaling up a prevention approach based on opportunistic screening, identification of a high-risk individual, treatment with a multi-drug regimen, and following a moderate increment in health expenditure (Lim et al., 2007). A simple FDC can help tackle CV health disparities by possibly improving the patients' adherence to medication and reducing the need for dose adjustments and multiple clinic visits (Muñoz and Wang, 2019). The CVPs can be a cost-effective, feasible, and overall attractive option in the improvement of CV health than

targeting individual risk factors (Yusuf, 2002; Lim et al., 2007). In the long term, these can help in reducing the healthcare costs (Roebuck et al., 2011; Becerra et al., 2015; Coca et al., 2023). Based on a wide range of factors, the cost of polypill has been anticipated between $0.06-$0.94/day (Singh et al., 2018). Studies have further provided evidences on cost-effectiveness of the recently trialled CNIC-Polypill (Becerra et al., 2015; Cordero et al., 2021). The economic analyses in the recent NEPTUNO study carried out on 6,456 patients in Spain has shown that the CNIC-Polypill used for secondary CV prevention is cost-effective compared to monotherapy and its use incurs a relatively lower utilization of the healthcare resources and total costs (González-Juanatey et al., 2022; Cordero et al., 2023). This further provided information on the associated cost-savings ranging between €17,790 to €26,257 per patient without CVE. This model reflected on significant increments in the patients' living years and quality of life. Furthermore, an economic model developed under the Portuguese MERCURY study evaluated the cost-effectiveness and public health benefits of the CNIC-Polypill compared to other alternative therapies (Aguiar et al., 2022).

Implementation of the Polypill therapeutic approach can be challenging, especially in the LMICs, given the constraints of low income, multiple clinical visits, testing and dose adjustments, and under-insurance of individuals in these countries. But these challenges are somehow manageable. The governments and policymakers in individual LMICs and other countries must ensure relatively easy access and ample supply of CVPs at nominal prices to the eligible individuals in their countries. On the other hand, the use of polypills can further be limited by eligibility determinants regarding polypill users. They should be above 55 years and have a history of CVD, systolic blood pressure ranging between 120 and 160 mmHg, LDL cholesterol <190 mg/dL, a glomerular filtration rate of at least 60 mL/min, less hepatic amino-transferase levels, normal potassium level, no contradictions to the polypill components, and using no more than two antihypertensive medications (Muñoz et al., 2019; Castioni et al., 2021). However, the use of CVPs in children and pregnant women as a preventive therapy is not recommended as some of the drug compositions (such as ACE inhibitors, anti-hypertensive drugs, statins) are contraindicated for their use (Alexander et al., 2009; JCS Joint Working Group, 2014; Kaelber et al., 2016; Mehta et al., 2020).

7 Tailoring the dose of individual polypill components

Recently in clinical practice, there has been a rising interest in precision medicine, particularly in pharmacogenetics and implementation of the genomic medicine. Studies have demonstrated a significant inter-individual variability in terms of drug response, which has been mainly associated with their specific genetic profiles or genotypes of CVD-related Pharmacogenes, influencing drug metabolism, drug transport, and drug effects (Vrablik et al., 2021). Lately, technological advancements have ushered the field of Pharmacogenetics and Pharmacogenomics and enabled the scientific community to correlate genetic variations with the efficacy and/or toxicity of anti-CVD drugs, facilitating the identification of individuals who could have a better response or are poor responders at a greater risk of developing adverse responses to a particular pharmacotherapy. Identification of such pharmacogenetic variants would further allow the consumption of relevant drugs at optimal dosing, thereby, improving the health outcomes of CVD patients. Although seems challenging, tailored dosing of individual drug components according to the patient's genetic profile would be intriguing and effective in enhancing the therapeutic outcomes of polypills. For further reference, information on some of the genetic variants associated with individual drug compositions of clinically trialled polypills and their responses is provided in Supplementary Table S1. Though in the context of personalized medicine, it is still questionable whether fine-tuning the polypill components should be population-specific or personalized. However, this would require further shreds of evidence.

8 Future directions

The use of CVPs in the preventive management of CVDs is on the rise lately. These are considered safe and highly effective secondary preventive medications for reducing the incidence of major CVDs. Studies have shown that three-quarters of the incidence of heart attacks and strokes could be averted if these are taken regularly for a longer period. Since the polypills have a simple regimen as a single-pill FDC, these have been proven beneficial in improving patients' adherence to medication with better clinical outcomes. CVPs have extensive potential of reducing the burden of CVDs, especially in LMICs, because of their cost-effectiveness and lesser side effects. The CVPs could correspond to a strategic therapeutic solution in hypertension, co-morbid, and non-adherent patients, even though further studies are required to understand their role in clinical practice. Though limited in number, recent clinical trials evaluating the efficiency of these CVPs have proven instrumental in providing information on their effectiveness in disease management and patient compliance to medication as well as their safety. Yet further studies are required to assess the impact of polypill-based therapy on hard clinical outcomes of CVD such as mortality and MACEs. Although CVPs are being considered for inclusion into the WHO List of Essential Medicines, there is still a long way to fully assess their potential in alleviating CVD-associated risk factors. Scaling up the polypill-based intervention is essential for reducing CVD-related mortality in the coming years. Demonstrating the potential health effects of CVPs and the cost of scaling up the required resources would influence relevant agencies to set an appropriate action plan to mobilize essential resources by developing necessary financial as well as infrastructural aids. At the most, the success of the recent clinical trials should motivate a large-scale implementation of polypills with all possible outcomes. To have a consensus on tailored dosing of individual polypill components, nevertheless, further evidence from more population-specific clinical trials targeting a larger population based on the genotype of relevant Pharmacogenes is a need-of-the hour. Maintaining a positive mindset in the public interest that does not impede the availability of cost-effective health interventions due to personal profit issues would greatly improve the outcomes of CVP-based intervention worldwide, especially for the LMIC populations. As Lonn et al. (2010) proposed, an individual high-risk intervention strategy should complement a population-based intervention approach to reduce risk factors in an entire population (Lonn et al., 2010). Ensuring patients' access to affordable preventive medications

like CVPs at a global level will substantially benefit them by improving their clinical outcomes.

In conclusion, polypill has a colossal clinical outcome in the primary and secondary management of CVD patients compared to monotherapies, despite a few complications associated with potential side effects of their individual medicinal compositions. These have proven to enhance adherence rates and lower healthcare costs, but more research is still needed to determine their safety and efficacy. Further studies are warranted to determine whether polypills can be custom-tailored for personalized therapeutics.

Author contributions

SB: Conceptualization, Supervision, Writing–review and editing, Data curation, Formal Analysis, Investigation, Validation, Writing–original draft. AA: Conceptualization, Data curation, Formal Analysis, Investigation, Project administration, Supervision, Writing–original draft, Writing–review and editing. AM: Conceptualization, Data curation, Writing–original draft, Writing–review and editing. RVS: Conceptualization, Data curation, Writing–original draft, Writing–review and editing. SVS: Conceptualization, Writing–review and editing, Data curation, Writing–original draft. MM: Conceptualization, Supervision, Writing–review and editing, Validation: SKS: Conceptualization, Writing–review and editing, Formal Analysis, Supervision, Validation. PV: Conceptualization, Writing–review and editing, Project administration, Resources, Supervision. AP: Conceptualization, Supervision, Writing–review and editing, Funding acquisition, Project administration, Resources.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. We acknowledge the Department of Biotechnology, India for supporting the study by providing the financial grant "Boost to University Interdisciplinary Life Science Departments for Education and Research" (DBT-BUILDER) via grant letter BT/INF/22/SP43045/2021, dated 22/11/2021.

Acknowledgments

The authors acknowledge the JSS Academy of Higher Education and Research (JSSAHER), Mysuru for providing the necessary research infrastructure and resources.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1268119/full#supplementary-material

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frontiers | Frontiers in Genetics

TYPE Brief Research Report
PUBLISHED 28 November 2023
DOI 10.3389/fgene.2023.1308738

OPEN ACCESS

EDITED BY
Francesca Coperchini,
University of Pavia, Italy

REVIEWED BY
Dennis Cheek,
Texas Christian University, United States
Marc Williams,
Geisinger Health System, United States

*CORRESPONDENCE
Sean P. David,
spdavid1@uchicago.edu

RECEIVED 07 October 2023
ACCEPTED 06 November 2023
PUBLISHED 28 November 2023

CITATION
David SP, Dunnenberger HM, Choi S, DePersia A, Ilbawi N, Ward C, Wake DT, Khandekar JD, Shannon Y, Hughes K, Miller N, Mangold KA, Sabatini LM, Helseth DL, Xu J, Sanders A, Kaul KL and Hulick PJ (2023), Personalized medicine in a community health system: the NorthShore experience.
Front. Genet. 14:1308738.
doi: 10.3389/fgene.2023.1308738

Personalized medicine in a community health system: the NorthShore experience

Sean P. David[1,2,3,4]*, Henry M. Dunnenberger[3], Sarah Choi[3], Allison DePersia[3,4,5,6], Nadim Ilbawi[1,2], Christopher Ward[3], Dyson T. Wake[3], Janardan D. Khandekar[5,7], Yvette Shannon[3,5,6], Kristen Hughes[3], Nicholas Miller[3], Kathy A. Mangold[8], Linda M. Sabatini[8], Donald L. Helseth[3], Jianfeng Xu[3], Alan Sanders[9,10], Karen L. Kaul[4,8,11] and Peter J. Hulick[3,4,5,6]

[1]Department of Family Medicine, NorthShore University HealthSystem, Evanston, IL, United States, [2]Department of Family Medicine, University of Chicago Pritzker School of Medicine, Chicago, IL, United States, [3]Mark R. Neaman Center for Personalized Medicine, NorthShore University HealthSystem, Evanston, IL, United States, [4]Outcomes Research Network, NorthShore University HealthSystem, Evanston, IL, United States, [5]Department of Medicine, NorthShore University HealthSystem, Evanston, IL, United States, [6]Department of Medicine, University of Chicago Pritzker School of Medicine, Chicago, IL, United States, [7]Kellogg Cancer Center, NorthShore University HealthSystem, Evanston, IL, United States, [8]Department of Pathology, University of Chicago Pritzker School of Medicine, Chicago, IL, United States, [9]Center for Psychiatric Genetics, Department of Psychiatry and Behavioral Sciences, NorthShore University HealthSystem, Evanston, IL, United States, [10]Departments of Psychiatry and Behavioral Neuroscience, University of Chicago Pritzker School of Medicine, Chicago, IL, United States, [11]Department of Pathology, NorthShore University HealthSystem, Evanston, IL, United States

Genomic and personalized medicine implementation efforts have largely centered on specialty care in tertiary health systems. There are few examples of fully integrated care systems that span the healthcare continuum. In 2014, NorthShore University HealthSystem launched the Center for Personalized Medicine to catalyze the delivery of personalized medicine. Successful implementation required the development of a scalable family history collection tool, the Genetic and Wellness Assessment (GWA) and Breast Health Assessment (BHA) tools; integrated pharmacogenomics programming; educational programming; electronic medical record integration; and robust clinical decision support tools. To date, more than 225,000 patients have been screened for increased hereditary conditions, such as cancer risk, through these tools in primary care. More than 35,000 patients completed clinical genetic testing following GWA or BHA completion. An innovative program trained more than 100 primary care providers in genomic medicine, activated with clinical decision support and access to patient genetic counseling services and digital healthcare tools. The development of a novel bioinformatics platform (FLYPE) enabled the incorporation of genomics data into electronic medical records. To date, over 4,000 patients have been identified to have a pathogenic or likely pathogenic variant in a gene with medical management implications. Over 33,000 patients have clinical pharmacogenomics data incorporated into the electronic health record supported by clinical decision support tools. This manuscript describes the evolution, strategy, and successful multispecialty partnerships aligned with health system leadership that enabled the implementation of a comprehensive

personalized medicine program with measurable patient outcomes through a genomics-enabled learning health system model that utilizes implementation science frameworks.

KEYWORDS

personalized medicine, precision medicine, precision health, genetic counseling, genetic testing, pharmacogenomics, primary care

Introduction

Since the completion of the Human Genome Project in 2003, the National Human Genome Research Institute (NHGRI) and other thought leaders set a bold vision to apply the advancement of genomic knowledge to address grand challenges in public health. The Grand Challenge II-5 investigated how genomic risk information is conveyed in clinical settings, how that information influences health strategies and behaviors, and how these affect health outcomes and costs (Collins et al., 2003). Twenty years later, the NHGRI's strategic vision is to advance 'virtuous cycles in human genomics research and clinical care' between innovative genomics research, genomic learning healthcare systems, and new knowledge generation "to improve health at the forefront of genomics" (Green et al., 2020).

NorthShore University HealthSystem is an integrated healthcare delivery system including nine hospitals and a multispecialty group practice with over 140 Chicagoland locations. NorthShore accepted the grand challenge of advancing genomics into health (Green et al., 2011), starting with a feasibility study involving primary care and specialty physicians about their interest in and preparedness for offering genomic services (Selkirk et al., 2013). Results indicated perceived low levels of confidence to provide genomic services, while patient demand for these services was increasing.

NorthShore launched a decade-long investment to build an innovative, integrated, personalized medicine program grounded within a rapidly learning healthcare system model. The Center for Personalized Medicine (PMED), launched in 2014, has a strategic focus on primary care clinical implementation and research. We describe our efforts as focused on germline genomics, addressing risk assessment, stratification, and scaling personalized care in a seamless, sustainable manner across a community health system. The goal is to deliver on the promise of personalized medicine by utilizing genomics to preempt illness and precisely treat disease to improve the health of our patients and families.

Methods

Early vision and foundation for personalized medicine

PMED's vision is to solve the "last mile" of implementation. The foundation began in 1989 with a "Molecular Biology and Genetics" task force to address the challenges for a future of genomics-guided care. The "Molecular Medicine" model had four core elements: clinical genetics, screening, diagnostics (molecular/cytogenetics), and research. Molecular Pathology (1992) and the Division of Genetics (1997) created a critical foundation. Strong partnerships between clinical and administrative champions were key for continued success through this journey (Figure 1).

Personalized medicine clinical and research infrastructure development

NorthShore invested in an institutional research biobank, the Genomic Health Initiative (GHI), that conducts translational, discovery research driven by the intersection of the electronic health record (EHR) and genomics data. Over 50,000 subjects have enrolled, and 30,000 DNA samples have been collected since 2014. The GHI's key operational learnings include using the EHR patient portal to streamline participant outreach, education, consenting, and automating blood collection at the next phlebotomy appointment without impeding primary care. The high and signaled community interest in the GHI indicates a growing interest in genomics and health. The GHI's participant outreach has enabled NorthShore to be a major partner in the Illinois Precision Medicine Consortium, which recruits participants for the National Institutes of Health (NIH)'s All of Us Research Program.

The development and clinical implementation of polygenic risk score (PRS) is a core translational research focus of PMED. Initial efforts focused on prostate cancer PRS and its clinical validity in multiple ancestral populations (Shi et al., 2021; Shi et al., 2022) and then expanded to additional conditions (Ahmed et al., 2022; Wei et al., 2022a; Glaser et al., 2022; Billings et al., 2023; Patel et al., 2023; Shi et al., 2023), including other cancer types (Shi et al., 2019; Northcutt et al., 2021; Wei et al., 2022b). Recently, our efforts have centered on the clinical implementation of PRS.

The Program for Personalized Cancer Care (PPCC) was implemented to improve the quality of cancer care through proactive, personalized care, from cancer prevention and screening to customized treatment of localized and advanced cancer with a focus on prostate cancer care. The Kellogg Cancer Genomic Initiative (KCGI) is a multidisciplinary program focused on the implementation and incorporation of NGS technology to better molecularly characterize cancer. Both are translational programs bridging the bench-to-bedside gap.

A pharmacogenomics (PGx) clinic (2015) was established with a multidisciplinary team to oversee the quality of testing, clinical guidance, and implementation, as described elsewhere (Dunnenberger et al., 2016). Scaling pharmacogenomics into primary care was an early priority given clinicians could relate to the concept that medications might work differently based on one's genetics and privacy concerns were minimal. PCPs were offered testing through a pilot program to gain experience before broader implementation. Patient and physician experiences were evaluated,

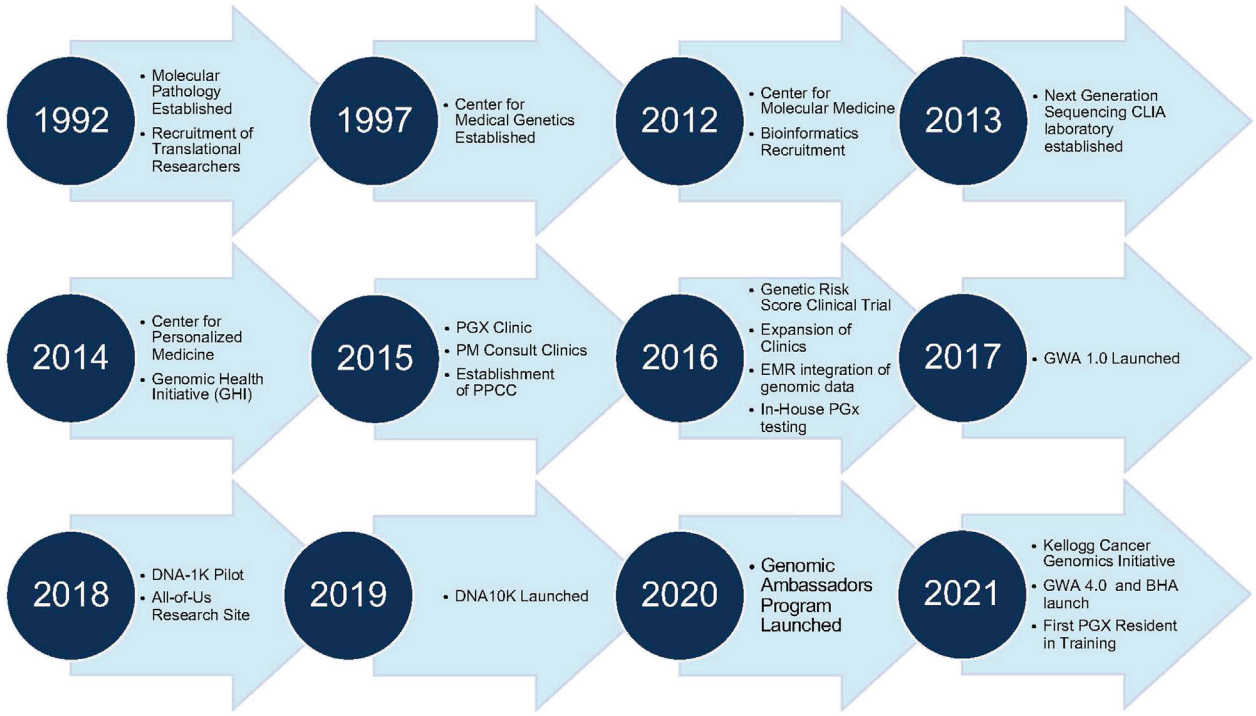

FIGURE 1
Evolution of personalized medicine at NorthShore. Timeline of Center for Personalized Medicine Developmental Milestones. BHA, Breast Health Assessment; CLIA, Clinical Laboratory Improvement Amendments of 1988; and GWA, Genomic and Wellness Assessment.

which provided critical learnings for scaling genomics in primary care (Lemke et al., 2017; Lemke et al., 2018).

Development of FLYPE and EPIC integration

FLYPE is an in-house web-based bioinformatics platform that addresses challenges with the integration of personalized medicine (Helseth et al., 2021). FLYPE maintains NorthShore's clinical knowledge base and updates variant annotations as new scientific knowledge emerges. Further integration into patients' EPIC (Verona, WI) EHRs and their clinical decision support (CDS) capabilities has been implemented system-wide. The genomic indicator (GI) functionality of EPIC is one mechanism for capturing clinically significant variant data (e.g., pathogenic germline variants and pharmacogenetic variants) and their clinical implications and for triggering CDS alerts. The educational CDS messaging was previously only available to healthcare providers, but it has recently been "turned on" for patients as well to improve their knowledge and engagement with genomic data.

Over 702,000 GIs have been assigned to over 38,000 patients for CDS. Pharmacogenomics represents the largest GI dataset (over 695,000 indicators) assigned. As of 1 August 2023, over 390 CDS PGx rules are utilized, powered by 165 different PGx GIs covering 50 genes supporting the Food and Drug Administration, Clinical Pharmacogenomics Implementation Consortium (CPIC), and the Dutch Pharmacogenetics Working Group guidance. CDS embedded in the EHR has been a focal point for education and informing patients and clinicians about clinical advances in the field (Pritchard et al., 2021).

Genetic and Wellness Assessment

PMED engaged widely with physician stakeholders for the implementation of direct access to PGx testing through primary care (Lemke et al., 2017). This crucial engagement laid a foundation for an EHR-integrated family health history tool, the Genetic and Wellness Assessment (GWA). The GWA began with a community educational event that invited participants, whether a NorthShore patient or not, to learn about personalized medicine. A simple "yes/no" questionnaire covering key core National Comprehensive Cancer Network® (NCCN) indications for hereditary cancer risk was provided. Many elected to pursue follow-up, which signaled the need to improve access and awareness around hereditary risk assessment in our community.

The GWA assesses personal/family history at one's annual history and physical appointment (Figure 2). Through the

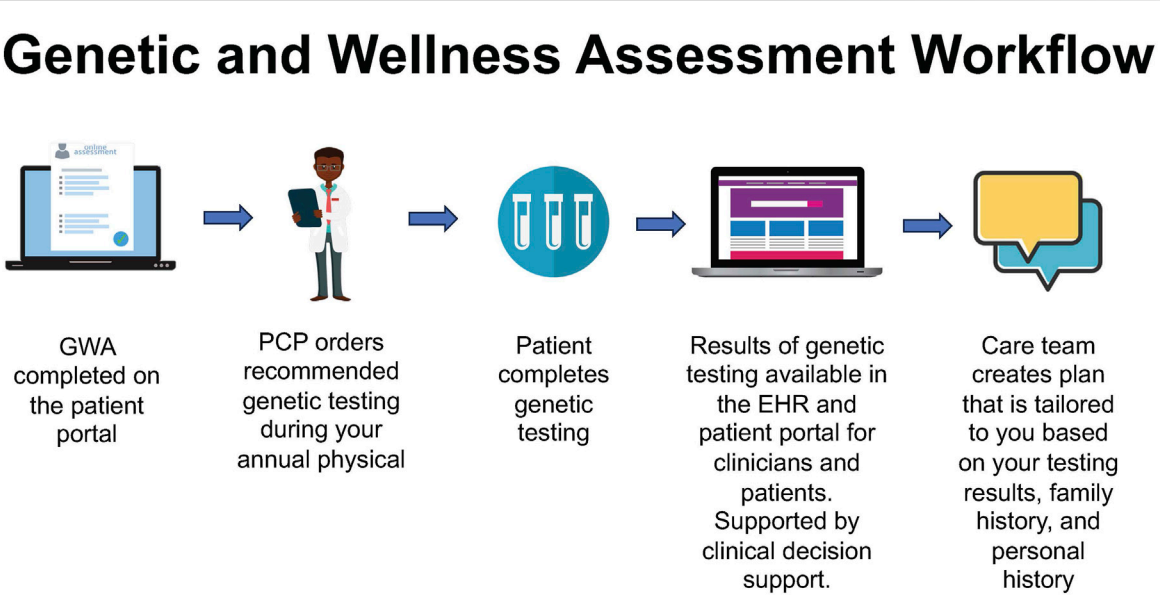

FIGURE 2
Genomic and Wellness Assessment workflow, starting with patient-facing registration and history, proceeding to primary care provider test ordering and clinical decision support, resulting in the return of results and development of a tailored care plan.

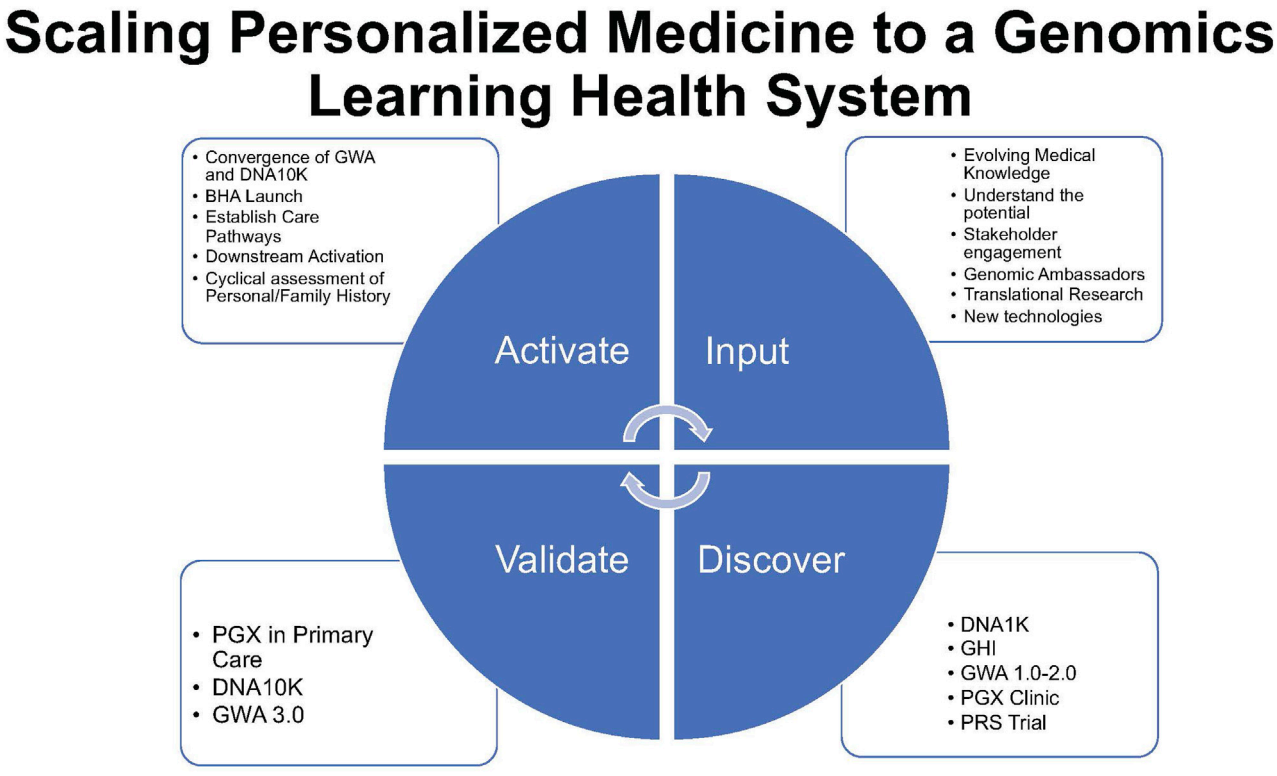

FIGURE 3
Scaling personalized medicine to a genomics learning health system represents a virtuous circle of activation, input of innovation, validation, adoption, adaptation, and renewal (activate–input–discover–validate) of the NorthShore implementation model.

electronic check-in appointment process, patients complete the GWA along with routine pre-visit items (e.g., verifying health insurance, medications, and depression screening). Having the GWA as part of the routine, standard workflow was a goal. The GWA provides evidence-based guidance for genetic testing by identifying patients at higher risk for inherited conditions. The

GWA has undergone a series of iterations guided by stakeholder feedback following the model of a learning healthcare system (Wouters et al., 2020) (Figure 3).

GWA 1.0 (2017) was the broadest iteration with 30 "yes"/"no" screening questions, with branching logic related to inherited cancer risk, cardiology, neurology, and endocrinology. Answers were prepopulated from EHR data. CDS alerts provided educational information in the line of care, prompting PCPs to consider genetic testing (e.g., hereditary cancer panel) or specialty referral or consideration of a "healthy gene panel" if targeted testing was not indicated. Specific specialists have been identified as genomics champions and content experts (Hulick et al., 2018). GWA 1.0 relied on physicians to act on Best Practice Alerts (BPAs), which had concise information regarding why an alert was fired and the rationale for the next step to be considered (e.g., referral and genetic testing). An internal review of 2018 data noted a 22% action rate on over 50,000 BPAs. Stakeholder feedback identified the following challenges: time necessary to complete the GWA, workflow as patients could fill out a paper alternative, and patient understanding of questions. Benefits included improved access to family history assessment and genetics. Based on the insights generated, iterative changes were made to the GWA (Lemke et al., 2020a). These included operational improvements with an emphasis on pre-visit workflows, reduction of the total number of questions, and limiting the domains to cancer and cardiovascular conditions. The GHI electronic consent and automated blood draw order signed by the principal investigator were implemented. BPA information was expanded based on the National Comprehensive Cancer Network® (NCCN) guidelines. Following the successful pilot of the two medical group sites, full medical group deployment of GWA 2.0 was carried out.

Population offering of genetic and pharmacogenomic screening (DNA1K and DNA10K)

While primary care physicians valued the GWA's clinical utility, they recognized the limitations of an approach based solely on family history (Lemke et al., 2020b). The GWA (1.0–2.0) remained labor intensive, given a requirement to "act on" a BPA. A more patient-driven, "easy button" approach was piloted to remove traditional genetic testing barriers: cost, gating testing based on family/personal history, and time constraints of a primary care clinic (Supplementary Figure S1). Key to an "easy button" approach was stakeholder agreement that informed consent be captured electronically prior to the appointment.

To assess interest, system feasibility, and patient outcomes, as part of a learning healthcare system initiative, complimentary hereditary cancer genetic testing (30-gene next-generation sequencing-based panel) was offered through four primary care sites during any clinic visit, ranging from an acute illness visit to a patient's annual exam. A total of 1,006 patients underwent testing in November and December of 2018 through DNA1K. An EHR review of these patients found that 92 pathogenic variants were identified in 90 (9.1%) patients in 16 genes (*MUTYH, CHEK2, APC, BRCA2, BRCA1, ATM, BARD1, MITF, BRIP1, NBN, PALB2, PMS2, RAD51C, MSH6, CDH1,* and *CDKN2A*). The high yield of positive tests and uptake of cancer screening test recommendations demonstrated the potential clinical utility of population-based screening programs. More patients completed genetic testing in 2 months than in the 18 months prior to the GWA (Supplementary Figure S2). DNA1K resulted in the launch of a coordinated care center to facilitate efficient patient follow-up through medical referrals and recommendations and the creation of a PCP advisory group ("Genomic Ambassadors"), and mechanisms were created to provide continual quality improvement and operational feedback to providers (Lemke et al., 2019).

NorthShore scaled DNA1K to DNA10K at 13 NorthShore Medical Group primary care sites spanning the health system's geography. This was a pragmatic shift to focus on "proactive" screening rather than relying on "reactive genetic testing" based on personal or family history. Personalized medicine strategies were systematically implemented within primary care through DNA10K, thereby streamlining processes and physician practices (David et al., 2021a). Over 100 PCPs ordered Color Extended test panels for more than 10,000 patients who participated from early 2019 through 2020. Patients had the option to gain access to "fun traits" related to genomics, including ancestry, through Color's patient portal to better understand the influence of direct-to-consumer offerings in the genomics space. Patients' experiences with DNA10K were highly positive (Lemke et al., 2021). A subset of medical group primary care sites not part of DNA10K piloted further refinements of GWA 2.0, leading to GWA 3.0, which included "A/B" testing of customer relationship management (CRM) messaging prior to a patient's visit, narrowing the line of questions to hereditary cancer, and development of a "more information" option for the patient to select after completing the GWA. This approach provided a method to schedule a complimentary genetic counseling assistant, a new position created based on feedback from primary care, visit under the supervision of the PMED team. This helped provide targeted discussion and triage of patients while relieving some of the burden on primary care physicians.

As a result of the iterative learnings from GWA 2.0 and DNA10K, the programs were merged to create a uniform primary care experience in January 2020, GWA 3.0 (Pritchard et al., 2021). Modeling the GHI consenting process and experience, patients were able to consent for testing with an order automatically placed in the PCP order set for the patient encounter. If the PCP did not override the order, it was automatically signed at the close of the EHR patient encounter and then released for sample collection and testing. To improve sustainability and clinical coverage, in GWA 4.0, patients who had a personalized or family history of cancer that likely met insurance coverage requirements were offered a hereditary cancer panel billed to insurance by our laboratory partner. Other patients were offered a population genetic screen with no insurance billing.

The GWA tool has undergone several refinements and has become more focused on hereditary cancer risk. The tool's clinical validity is the highest when accessing this risk, and the gene content has been adjusted accordingly. The Breast Health Assessment (BHA), which is an adaptation of the GWA, focuses on the hereditary risk of breast and ovarian cancer types and is offered with screening mammography to capture a larger fraction of the population potentially at risk for cancer.

10.3389/fgene.2023.1308738

TABLE 1 Demographics by version of the GWA and BHA.

	GWA 1.0	GWA 2.0	GWA 3.0	GWA 4.0	BHA
Total patients (N)	52,902	31,091	87,575	153,995	33,609
Race, n (%)					
American–Indian or Alaska native	140 (0.3)	92 (0.3)	193 (0.2)	393 (0.3)	63 (0.2)
Asian	4,311 (8.1)	2,636 (8.5)	6,837 (7.8)	14,888 (9.7)	2,651 (7.9)
Black or African–American	2,458 (4.6)	1,842 (5.9)	3,614 (4.1)	6,602 (4.3)	1,707 (5.1)
Pacific Islander or Hawaiian native	0 (0)	46 (0.1)	94 (0.1)	173 (0.1)	30 (0.1)
Other or unknown	10,623 (20.1)	6,950 (22.4)	16,168 (18.5)	29,065 (18.9)	5,239 (15.6)
White	35,315 (66.8)	19,525 (62.8)	60,669 (69.3)	102,874 (66.8)	23,919 (71.2)
Ethnicity, n (%)					
Hispanic/Latino	3,344 (6.3)	2,772 (8.9)	5,480 (6.3)	11,803 (7.7)	1,772 (5.3)
Non-Hispanic	49,127 (92.9)	28,037 (90.2)	81,316 (92.9)	14,0376 (91.2)	31,640 (94.1)
Unknown	431 (0.8)	282 (0.9)	779 (0.9)	1,816 (1.2)	197 (0.6)
Age, n (%)					
18–39	15,114 (28.6)	9,804 (31.5)	22,464 (25.7)	44,713 (29)	268 (0.8)
40–49	10,778 (20.4)	6,296 (20.3)	15,440 (17.6)	28,287 (18.4)	7,990 (23.8)
50–64	20,386 (38.5)	11,767 (37.8)	26,681 (30.5)	43,699 (28.4)	13,773 (41)
65+	6,624 (12.5)	3,224 (10.4)	22,990 (26.3)	37,296 (24.2)	11,578 (34.4)
Sex, n (%)					
Male	20,506 (38.8)	10,389 (33.4)	32,562 (37.2)	58,930 (38.3)	4 (0)
Female	32,369 (61.2)	20,702 (66.6)	55,013 (62.8)	95,060 (61.7)	33,605 (100)

The current GWA version 4.0 (and analogous BHA) has been completed by over 150,000 individual patients, over 30,000 patients have screened "positive" on the personal/family history collection, and over 7,800 have undergone genetic testing through this primary care initiative. Similar success has been achieved with the BHA, with over 33,000 patients completing, 8,000 screening positive, and 2,300 patients pursuing genetic testing (Table 1). More patients have undergone genetic testing and risk assessment with the current GWA/BHA versions than in the history of the division of genetics over multiple decades (Supplementary Figure S1).

Education and accelerating the diffusion of knowledge

Accelerating the diffusion of knowledge regarding the incorporation of genomics into medical care is critical for the success of the program. Educational videos were created for internal and external audiences covering key topics ranging from GINA and pharmacogenomics to downstream implications on screening and management. Virtual town halls were created for Q&A along with more traditional educational outreaches, such as grand round lectures and "lunch and learn" sessions for primary care offices.

Establishing the Genomic Ambassadors program enabled PMED to innovate quickly and disseminate knowledge. Approximately 10 primary care providers per year are supported by PMED to assist with generating insights, developing improvement strategies, implementing strategies, and then, reviewing data to determine success. This targeted model of a learning health system has led to ongoing incremental improvements to the program. It further amplifies the "voice" of our genomics content experts in our systems as the primary care ambassadors teach and receive input from their peers as part of the program.

Expansion of population screening to diverse communities

The NorthShore merger with Swedish Covenant Hospital in 2020 expanded the patient catchment area to northern Chicago and provided an opportunity to expand access to an ethnically and socioeconomically diverse patient population. Since the expansion, thousands of Swedish Hospital patients have completed the GWA, and the PMED team has engaged in community-based participatory research with more diverse communities (Lemke et al., 2022).

Results

Mixed-methods research to better understand the patient and provider experiences

To lay the foundation for wider implementation of a PGx program, PMED investigators employed multiple methods to query PCP readiness and the patient experience. Three main themes emerged: perceived value and utility of PGx testing, challenges to implementation in practice, and provider and patient needs. PCPs expressed perceived benefits of PGx testing, such as avoiding adverse drug effects, more efficient dose titration, improved shared decision making, and the ability to provide patients with reassurance. Concerns were expressed about the privacy, cost, insurance coverage and level of knowledge regarding PGx results (Lemke et al., 2017). Patients also expressed no difference in the personal utility of PGx testing offered through a designated PGx clinic or with direct in-home testing. However, some did express privacy concerns, and most were unfamiliar with privacy protections provided by the GINA Act (Lemke et al., 2018).

Four main themes emerged regarding GWA implementation: benefits to clinical care, challenges in practice, CDS-specific issues, and physician-recommended improvements. Sub-themes emerged, such as perceived value in increased access to genetic services, time limitations to discuss GWA recommendations, lack of patient adherence with recommendations, and provider alert fatigue. These findings suggested that while PCPs valued the clinical utility of the GWA, there remained several challenges identified with its administration and use in practice (Lemke et al., 2020a).

A mixed-methods approach assessed PCP readiness and the patient experience with DNA10K. Like their PGx and GWA experiences, PCPs expressed concerns about and limited confidence with tasks related to test ordering, interpretation, and management of the results. Respondents perceived a high level of clinical utility for patients and their families, though there were logistical challenges to incorporating testing into their busy practices. PCPs were also unfamiliar with the privacy protections of the GINA and were concerned about patient data privacy and the potential for insurance discrimination. Adaptive refinements of several processes were implemented that improved the PCP experience with DNA10K (Lemke et al., 2020b). To assess the patient's experience with the deployment of DNA10K, patients were offered an online survey 3 weeks after result disclosure and in six months. The patient reported understanding of results was high for cancer and cardiovascular disease risk variants. The overwhelming majority of patients perceived its personal utility as "high," most patients shared the results with their families, and most patients expressed high levels of satisfaction with the process. Moreover, result-related health behaviors and discussions with PCPs increased over time, particularly for patients with "positive" test results (Lemke et al., 2021).

Genetic screening and testing outcomes for GWA and DNA10K

As of 1 August 2023, a version of GWA (1.0-4.0) or BHA has been completed at least once by 228,766 patients (Table 1), with 35,432 patients completing genetic testing within NorthShore after having completed either the GHA or BHA. A total of 4,662 pathogenic (P) or likely pathogenic (LP) variants have been assigned to 4,084 patients with 463 P/LP variants in Centers for Disease Control & Prevention (CDC) Tier 1 conditions (HBOC, Lynch syndrome, Familial Hypercholesterolemia).

Pharmacogenomics and medical outcomes

PMED's growing PGx program and its relationship with the NorthShore Outcomes Research Network have made possible the evaluation of medical outcomes, including hospital admissions, readmissions, and analyses of the relationship between multimorbidity, polypharmacy, social determinants of health, and gene–drug interactions. David et al. first reported that DNA10K patients (n = 10,104) were significantly more likely to be readmitted within 90 days of hospital discharge if they had one or more PGx interactions with CPIC medications prescribed within 30 days of admission (odds ratio (OR) = 1.42, 95% confidence interval (CI) 1.09–1.84 (p = 0.01)). After adjustment for comorbidities and other covariates, the odds of readmission were increased by more than 30% for patients with one or more CPIC PGx interactions (OR = 1.32, 95% CI 1.02-1.73) (p = 0.04) (David et al., 2021b). In a follow-up evaluation with roughly twice the sample size, led by Saulsberry and the PMED team, we replicated these findings and also showed that social determinants of health (including race, employment status, and income) were the major drivers of hospital readmission (Saulsberry et al., 2022). In fact, the odds of 90-day readmission for patients with one or more identified gene–drug interactions after adjustment for robust SDOH and other covariates was attenuated by 10% (OR = 1.31, 1.08–1.59) (p = 0.006). The PMED team is currently evaluating the relationship between the most widely prescribed CPIC mediations, gene–drug pairs, and condition-specific outcomes, and our team is exploring the use of natural language processing and machine learning to improve the efficiency and fidelity of data mining of the EHR to capture adverse drug events resulting from pharmacogenomic interactions.

Implementation of the polygenic risk score in primary care for personalized cancer screening

Leveraging research and clinical infrastructures at NorthShore, such as the GHI, large numbers of PCPs, and the EHR, we assessed the feasibility of the clinical implementation of the PRS in primary care. In a pilot study (Conran et al., 2021), we identified 281 subjects through the GHI who were 40–70 years old and without a personal history of breast, prostate, or colorectal cancer. The PRS for these cancer types was calculated and shared with participants through their primary care provider. Over 20% of these subjects received at least one high PRS for these three types of cancer. Many of these subjects did not have any known family history and otherwise would not realize their increased risk.

Discussion

Lessons learned from a decade of adoption and implementation

Starting with a needs assessment of physician stakeholders and expanding to mixed-methods and outcomes research, NorthShore's experience with patient-centered formative research has driven an iterative process of rapid adoption and implementation in a learning healthcare system. Iterative learning from patients, providers, and leadership and community stakeholders has fueled a virtuous cycle of activation, innovation, validation, adoption, adaptation, and renewal symbolized as "activate–input–discover–validate" (Figure 3). Sharing our experience is key to the advancement of clinical genomic medicine and is a foundation for the diffusion and implementation of future technologies.

There were challenges and critical inflection points in the evolution of PMED. Key lessons learned include ensuring there is continuous wide stakeholder engagement, periodic reassessment of the educational needs of patients and clinicians, and the importance of developing partnerships between clinical and administrative champions for PMED within the organization. Defining what personalized medicine meant at NorthShore was important in developing the roadmap. Initially, PMED focused on PGx and then evolved to germline risk assessment, which was complementary to pathology efforts in oncology. To ensure success, an organization needs to have administrative and clinical alignment on *what* they are implementing.

Educating and building acceptance in a targeted area of implementation is critical stakeholder buy-in. Primary care education on *why* genomics should be important to the busy primary care clinician was critical for PMED. At the core, tackling the concept of "genetic exceptionalism" was critical, and debunking the notion that genetics is "too complicated" or "esoteric" compared to other areas of medicine for primary care was necessary. Over time, as CDC Tier 1 conditions, NCCN, and U.S. Preventive Services Task Force (USPSTF) guidelines continue to expand, the importance of genomics and family history screening from a primary care perspective is becoming more defined.

Finally, understanding the business value proposition is paramount for long-term success. The balance of "fee for service" to "value-based" care will differ depending on the organization and, thus, change the inputs and outputs for a financial model for an organization contemplating a personalized medicine program. This can impact the decision on whether to implement first in primary care or within certain specialties, such as cardiology and oncology. The key deliverable is to bring the appropriate financial stakeholders to the discussion when exploring plans for a personalized medicine program.

Standing at the forefront of personalized medicine implementation, we seek to square up to a range of remaining challenges and implementation gaps. At each step of the GWA process, from completing the tool to follow-through on recommended screening and prevention, there is attrition. The balance between having an "easy button" solution *versus* one that requires more engagement of the PCP is critical as primary care endorsement was the most impactful driver to have a patient move to the next step. Keeping patients engaged and informed of their genetics over time poses additional challenges. While future testing may be required as technology evolves, the data currently available can provide insight over a lifetime and evolve with new medical knowledge. For example, the *NBN* c.657_661del (p.Lys219fs) variant has had changing NCCN recommendations regarding its impact on breast cancer risk (NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 2.2024) (Daly et al., 2021). Dissemination of this knowledge is now possible due to the infrastructure in place but remains challenging until more robust care pathways can be deployed at different touchpoints in the healthcare system.

We join with other learning health systems across the US to share successful personalized medicine adaptations that advance health equity and are transportable to other communities.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

SD: Conceptualization, Formal Analysis, Funding-acquisition, Investigation, Methodology, Supervision, Visualization, Writing–original draft, Writing–review and editing. HD: Conceptualization, Data-curation, Investigation, Project-administration, Software, Supervision, Writing–review and editing. SC: Resources, Writing–review and editing. AD: Conceptualization, Project-administration, Writing–review and editing. NI: Conceptualization, Project-administration, Writing–review and editing. CW: Data-curation, Formal Analysis, Methodology, Writing–review and editing. DW: Data-curation, Formal Analysis, Writing–review and editing. JK: Conceptualization, Funding-acquisition, Methodology, Resources, Supervision, Visualization, Writing–review and editing. YS: Project-administration, Writing–review and editing. KH: Project-administration, Writing–review and editing. NM: Software, Writing–review and editing. KAM: Writing–review and editing, Data-curation, Methodology, Project-administration, Validation, Resources. LMS: Writing–review and editing, Data-curation, Methodology, Project-administration, Validation, Resources. DH: Investigation, Methodology, Software, Writing–review and editing. JX: Formal Analysis, Investigation, Writing–review and editing. AS: Funding-acquisition, Project-administration, Supervision, Writing–review and editing. KK: Methodology, Project-administration, Supervision, Visualization, Writing–review and editing. PH: Conceptualization, Investigation, Methodology, Project-administration, Resources, Supervision, Writing–original draft, Writing–review and editing.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article. The DNA10K initiative is funded by the Transformation Through Innovation Fund at NorthShore University HealthSystem. The Center for Personalized Medicine receives some funding through donations to the NorthShore Foundation. Personal funding was granted to SPD from National Institutes of Health/National Center for Advancing Translational Sciences grant UL1-TR002389 and the NorthShore Auxiliary Research Scholar Award.

Acknowledgments

The authors thank Christina Herrington, Kamalakar Gulukota, Amy Lemke, Adam Matsil, Lavisha Singh, Jaclyn Maropoulos, Justin Brueck, Brian Helfand, Lauren Welles, Darryck Maurer, Milena Draganov, Bryce Hadsell, Susan Stasinos, Kathy Livschiz, Renee Birn, the Genomic Ambassadors, and other NorthShore Leadership members for their contributions.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2023.1308738/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 11 December 2023
DOI 10.3389/fphar.2023.1303844

OPEN ACCESS

EDITED BY
Alessio Squassina,
University of Cagliari, Italy

REVIEWED BY
Michele Mazzanti,
University of Milan, Italy
Yashmin Choudhury,
Assam University, India

*CORRESPONDENCE
Mario Rotondi,
mario.rotondi@icsmaugeri.it

RECEIVED 28 September 2023
ACCEPTED 30 November 2023
PUBLISHED 11 December 2023

CITATION
Greco A, Coperchini F, Croce L, Magri F, Teliti M and Rotondi M (2023), Drug repositioning in thyroid cancer treatment: the intriguing case of anti-diabetic drugs.
Front. Pharmacol. 14:1303844.
doi: 10.3389/fphar.2023.1303844

Drug repositioning in thyroid cancer treatment: the intriguing case of anti-diabetic drugs

Alessia Greco[1,2], Francesca Coperchini[1,2], Laura Croce[1,2], Flavia Magri[1,2], Marsida Teliti[1,2] and Mario Rotondi[1,2]*

[1]Department of Internal Medicine and Therapeutics, University of Pavia, Pavia, Italy, [2]Laboratory for Endocrine Disruptors, Unit of Endocrinology and Metabolism, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy

Cancer represents the main cause of death worldwide. Thyroid cancer (TC) shows an overall good rate of survival, however there is a percentage of patients that do not respond or are refractory to common therapies. Thus new therapeutics strategies are required. In the past decade, drug repositioning become very important in the field of cancer therapy. This approach shows several advantages including the saving of: i) time, ii) costs, iii) *de novo* studies regarding the safety (just characterized) of a drug. Regarding TC, few studies considered the potential repositioning of drugs. On the other hand, certain anti-diabetic drugs, were the focus of interesting studies on TC therapy, in view of the fact that they exhibited potential anti-tumor effects. Among these anti-diabetic compounds, not all were judjed as appropriate for repositioning, in view of well documented side effects. However, just to give few examples biguanides, DPP-4-inhibitors and Thiazolidinediones were found to exert strong anti-cancer effects in TC. Indeed, their effects spaced from induction of citotoxicity and inhibition of metastatic spread, to induction of de-differentiation of TC cells and modulation of TC microenvironment. Thus, the multifacial anti-cancer effect of these compounds would make the basis also for combinatory strategies. The present review is aimed at discuss data from studies regarding the anti-cancer effects of several anti-diabetic drugs recently showed in TC in view of their potential repositioning. Specific examples of anti-diabetic repositionable drugs for TC treatment will also be provided.

KEYWORDS

drug-repositioning, thyroid-cancer, anti-diabetic, metformin, diabetes

Introduction

Cancer mortality represents one of the most significant social, medical, and scientific primary challenges. The course of history teaches us that several effective anti-cancer drugs have been developed from ancestral compounds. The first employed anti-cancer drugs were poorly-selective (killing rapidly proliferating cells) and with high-spectrum (effective against most tumors). More new compounds have been added as possible tools for anti-cancer therapies, being characterized by a more selective mechanism and fewer side effects, resulting in better anti-cancer responses and/or fewer side effects. Nowadays, modern anti-cancer drugs are very selective (against a unique target) and, as a consequence, are effective only against tumor-bearing specific molecular abnormality (i.e., the higher selectivity, the lower antitumor spectrum). Recent advances in the knowledge of cancer biology led to the development of a new diagnostic and therapeutic arsenal that is not limited to selective

target drugs but also includes already existing drugs that are multi-targeted or used in polypharmacology (Gonzalez-Fierro and Dueñas-González, 2021).

Drug repositioning is considered for several types of cancer. The collection of information regarding the anti-cancer effects of drugs commonly used for other diseases could be of help in identifying potential repositionable compounds. As far as thyroid cancer (TC) treatment is concerned, few studies took into account the potential repositioning of drugs.

TC is the most common endocrine cancer, which has raised concern due to its rapidly increasing prevalence. Annual incidence varies by sex, age, and geographical location (Palanca et al., 2022). The incidence of TC is the ninth highest in the world (Chen DW. et al., 2023a) and, during the last 40 years, increased globally. According to many studies, the increase in TC incidence is a consequence of the detection of small, low-risk papillary TC due to increased thyroid ultrasonography use. During the past 5–10 years, TC care experienced profound changes, with several therapeutic options now available (Palanca et al., 2022; Chen DW. et al., 2023a).

Most TCs are slow-growing and highly responsive to standard therapies which include thyroidectomy, and in selected cases radioactive iodine treatment (RAI), and TSH suppressive therapy (Haugen et al., 2016). Even if these approaches are successful in many cases, there is still a subset (≈3%–5% of patients) that progress to therapeutically refractive disease, constituting the so-called "TC-related deaths due to the lack of effective treatment." In this view, some efforts are ongoing to identify personalized therapy for refractory patients and drug repositioning represents one of the potential alternatives (Xu et al., 2019). In particular, several studies suggested that some anti-diabetic drugs could be of potential interest because of their anti-cancer effects demonstrated both *in vitro* and *in vivo* on TC.

This review aims to overview the available studies regarding the multiple anti-cancer effects of anti-diabetic drugs discovered in TC as well as to discuss their potential repositioning for TC therapy. Moreover, an exhaustive description of drug repositioning including specific examples of anti-diabetic repositionable drugs for TC treatment will be also provided.

Drug repositioning

Drug repositioning is an alternative approach to identify new fields of application for existing drugs, currently approved for a different clinical condition. The drug repositioning activity is based on *in vivo* and *in vitro* tests. Polypharmacology is the basis for drug repurposing: this principle asserts that a drug with multiple targets can have multiple mechanisms of action (Nowak-Sliwinska et al., 2019). Therefore, polypharmacology can be used in the search for more effective and less toxic treatments.

Drug repositioning could indeed present several advantages as compared to the development of a new drug. Once repositioned, information regarding the safety, pharmacology, and toxicology of a given drug already exists, being approved by the Food and Drug Administration (FDA) for clinical use in humans.

Drug repurposing is time- and cost-saving compared with "*de novo*" drug development. Indeed, the time from discovery to clinical trial averages 9 years, the success rate is less than 10% with elevated average cost. In contrast, drug repositioning can take 3–4 years to reach clinical trials (Sams-Dodd, 2005). However, in this case, there are numerous obstacles to cross, mainly financial and resource barriers, intellectual properties, data access barriers, biases, and liability risks (Masuda et al., 2020).

Numerous collaborative initiatives with the final aim of drug repurposing were objects of debate, among wich: The AZ Open Innovation program, the NIH National Center for Advancing Translational Sciences (NCATS) program: Discovering New Therapeutic Uses for Existing Molecules, the Medical Research Council (MRC) and AstraZeneca (AZ) Mechanisms of Human Disease Initiative, The Clinical and Translational Science Award (CTSA) Pharmaceutical Assets Portal, European College of Neuropsychopharmacology (ECNP) Medicines Chest Program, Pfizer's SpringWorks Program, the AstraZeneca/National Research Program for Biopharmaceuticals (NRPB) partnership in Taiwan, the Roche/Broad Institute Collaboration, and the Drugs for Neglected Diseases Initiative (DNDI), the Clinical Development Partnerships Initiative (Krishnamurthy et al., 2022).

Some examples of drugs that have been repositioned for the treatment of certain types of cancer over the years include Sunitinib, a drug that was successfully repositioned for the treatment of gastrointestinal stromal tumors and renal cancers. In addition, in 2010, this compound was also approved for the treatment of pancreatic neuroendocrine cancers. Another drug Tamoxifen, that was originally known for its ability to increase fertility, was repurposed for breast cancer therapy. Moreover, it was proven to reduce breast cancer risk and approved also for this purpose. Tamoxifen was included in the standard of care for long-term adjuvant therapy for estrogen receptor-positive breast cancer (Krishnamurthy et al., 2022).

The main aim of drug repositioning is to counteract attrition and rising costs, which, according to "Eroom's law," have produced a large increase in the number of new drugs entering the pharmaceutical market. However, this approach must be considered as an add-on rather than an alternative to the search for novel drugs. (Jourdan et al., 2020).

In drug repositioning it is crucial to understand the disease-drug relationship, for this reason, there are several approaches to address the problem:

- In silico approaches: in this type of approach, various public databases, clinical trials, and bioinformatic systems are used to identify drug-target interactions (Ekins et al., 2007).
- Cluster approaches tend to find new drug-target or drug-disease relationships (Parisi et al., 2020).
- Propagation approaches: this approach allows the discovery of disease-gene, disease-disease, and disease-target relationships (Picart-Armada et al., 2019).
- Experimental approaches: including target screening, cellular assays (Shah et al., 2016), animal models (Ridges et al., 2012), and clinical studies (Zhu et al., 2014).

An important aspect of drug repositioning is to identify a compound that could also overcome the obstacle of drug resistance in cancer therapy. Therefore, it is necessary to follow

TABLE 1 Commonly used anti-diabetic drugs.

Classes of antidiabetcs	Name
Biguanides	-Metformin (in use)
	-Phenformin (removed in 1970)
	-Buformin (removed in 1970)
Sulfonylureas	-Glibenglamide (in use)
	-Glicazide (in use)
	-Glimepiride (in use)
Glinides	-Gepaglinide (in use)
Thiazolidinediones	-Troglitazone (removed in 2000)
	-Pioglitazone (in use)
	-Rosiglitazone (removed in 2010)
	-Ciglitazone (prototype, never used in clinical practice)
	-Lobeglitazone (in use only in korea)
DPP-4 enzyme inhibitors	-Sitagliptin (in use)
	-Gempigliptin (in use)
	-Linagliptin (in use)
	-Saxagliptin (in use)
	-Vildagliptin (in use)
	-Alogliptin (in use)
Inhibitors of the renal glucose transporter SGLT-2	-Canagliflozin (in use)
	-Dapagliflozin (in use)
	-Empagliflozin (in use)
Glp-1 analogues	-Exenatide (in use)
	-Liraglutide (in use)
	-Semaglutide (in use)
	-Dulaglutide (in use)
	-Lixisenatide (in use)
Insulin analogues	Fast acting
	- Lispro (in use)
	- Aspart (in use)
	- Glulisine (in use)
	Long acting
	- Detemir (in use)
	- Degludec (in use)
	- Glargine (in use)
	Intermediate acting
	- Human insulin (in use)
	- NPH (in use)

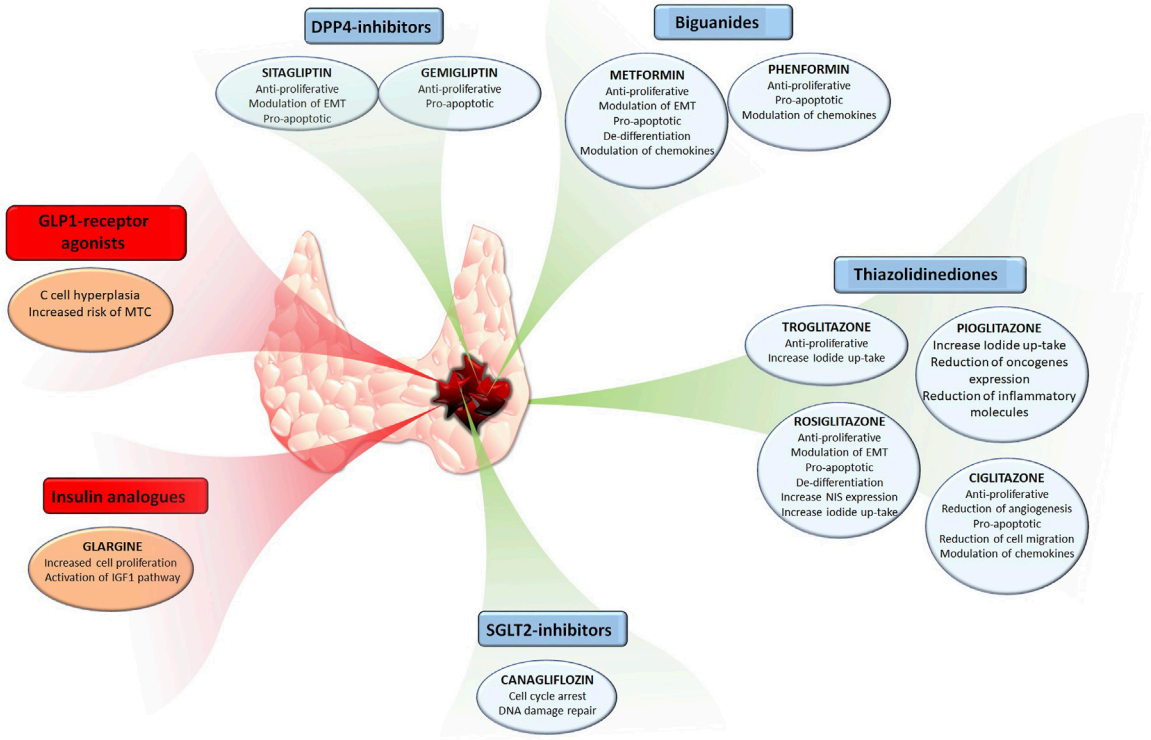

FIGURE 1
The effects of common anti-diabetic drugs in thyroid cancer.Anti-diabetic compounds exerting anti-cancer effects are have been reported in the blue boxes, also highlighting their respective effects. Anti-diabetic compounds exerting pro-tumorigenic effects are have been reported in the red boxes, also highlighting their respective effects.

strategies to minimize drug resistance and maximize antitumor activity (Wang et al., 2019).

Anti-diabetic drugs repositionable as potential anti-cancer drugs

Diabetes Mellitus (DM) is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood glucose. In type 2 diabetes mellitus (T2DM), hyperglycemia is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels. Hypoglycemic agents, also known as oral antidiabetics, represent a heterogeneous group of drugs that are used in the treatment of T2DM.

DM is characterized by elevated blood sugar (glycemic) levels, therefore the role of these drugs is to reduce glycemic levels, possibly avoiding hypoglycemic events. The other important aim of modern anti-diabetic therapy is to reduce mortality risk and long-term complications of T2DM, such as cardiovascular, renal and neurological damage. These drugs work by different mechanisms, some reduce blood glucose by increasing the secretion of insulin; others slow intestinal absorption of glucose, others increase renal elimination of glucose and others increase insulin sensitivity of target tissues. The classes of antidiabetics currently in use are summarized in Table 1.

The rationale behind the use of anti-diabetic drugs as potential candidates for drug repositioning in anti-cancer therapy, especially for TC, stems from several data. First of all, it is widely recognized that T2DM is a risk factor for developing several types of cancer, mainly colorectal, liver, pancreatic, and endometrial cancer (Ling et al., 2023). Some studies have also suggested a possible correlation between T2DM and TC (Aschebrook-Kilfoy et al., 2011; Yeo et al., 2014). Moreover, some authors have suggested that hyperinsulinemia, hyperglycemia, and chronic inflammation typical of untreated DM would be risk factors for TC development and/or progression (Jee et al., 2005; Giovannucci et al., 2010), and thus correcting them with anti-diabetic drugs would have indirect therapeutic effects for TC. Nevertheless, it must be acknowledged that some studies failed to report any association between T2DM and TC (Kitahara et al., 2012; Tse and ng, 2012; Seo et al., 2017). Moreover, the association observed in some epidemiological studies could be sustained by the fact that T2DM and TC could share some common risk factors, such as obesity and increasing age (Kitahara et al., 2020), and TC is often diagnosed incidentally in patients with T2DM due to the performance of screening imaging (such as carotid artery ultrasound), potentially leading to a selection bias (Croce et al., 2023).

The second reason why anti-diabetic drugs could be beneficial in TC is that TC cells often display abnormal metabolic pathways that

could be sensitive to the specific action of anti-diabetic drugs. In particular, malignant cells can modify their energy metabolism in response to a challenging environment, allowing tumor cells to survive and spread (Coelho et al., 2018). Most malignant cells rely on glucose metabolism through aerobic glycolysis, the so-called Warburg effect, with a sharp increase in glucose uptake and lactate production (Lin et al., 2020). This metabolic shift has also relevant consequences beyond favoring cancer growth, such as influencing tumor microenvironment and vascular invasion. Some anti-diabetic drugs can interfere with intracellular metabolic regulation, for example, through activation of AMPK or inhibition of glucose uptake, favoring TC cell death and also reducing its aggressiveness.

Biguanides

Biguanides are oral hypoglycemic drugs derived from the *Galega officinalis* plant, originally developed for the treatment of T2DM. The active components responsible for the effects were guanidines and galectins. In 1918, animal studies demonstrated that guanidine had hypoglycemic activity, but it was highly toxic for clinical use. Due to this reason, attention was focused on synthetic derivatives of these natural compounds. The first biguanide to be tested on humans in the XX century was metformin. In 1957, Jean Stern physician and clinical pharmacologist published the results of a study on the antidiabetic properties of several biguanides. Among them, dimethyl biguanide (known as metformin) was selected for clinical development with the suggestive name of "Glucophage," which means "glucose eater." The other biguanides, phenformin and buformin, were more potent than metformin, however in the late 1970s they were removed in most countries because of their association with lactic acidosis (Blough et al., 2015). Thus, among biguanides, metformin remains at present the most widely employed anti-diabetic drug. Of note, biguanides are not limited to be used as anti-diabetic drugs. Indeed, biguanide derivatives are used for multiple therapeutic purposes ranging from antimalarial (proguanil, cycloguanil), antiviral (monoxydine), anti-septic and disinfectant (chlorhexidine, alexidine, pycloxidine, polyhexanide) (Kathuria et al., 2021). Biguanides are extensively studied for their potential anti-cancer properties in several types of cancer including the thyroid one. The anti-cancer properties of biguanides are displayed through several mechanisms of action, including the activation AMPK pathway with the consequent inhibition of mTOR and reduction of several pro-tumorigenic effects including cell proliferation and production of inflammatory mediators (Seyfried et al., 2014; Luengo et al., 2017). Biguanides also interacts with REDD1, (Luengo et al., 2017), IRS receptors, (Luengo et al., 2017), glucose metabolism, (Luengo et al., 2017; IDF, 2021; WHO, 2023), oxidative phosphorylation, (Stine et al., 2022; Batta et al., 2020; Abudawood, 2019), lactic acid production, (Luengo et al., 2017), and cell cycle (Bell et al., 2023). Of note many other mechanisms are involved in the anti-cancer effects of biguanides, some of them more related to the specific cancer type, like, for example, the interaction of proguanil with EGFR in glioblastoma cells. Thus, due to the broad spectrum of action, biguanides are currently regarded as extremely interesting compounds.

Metformin

Metformin is an effective hypoglycemic agent, recommended as a first-line oral therapy for T2DM. Metformin was first approved in Canada in 1972, and received subsequent FDA approval in the United States in 1995. Metformin exerts its anti-diabetic effects through several mechanisms, such as inhibition of hepatic gluconeogenesis, reduction of insulin resistance, and enhancement of peripheral glucose uptake. Among its many possible pharmacologic properties, also anti-cancer effects have been reported. (Kushchayeva et al., 2014). Indeed, both *in vitro* and *in vivo* studies showed that metformin exerts benefits in several types of cancer. Metformin was shown to display anti-cancer effects through its insulin-lowering activity, which may slow tumor proliferation in individuals with insulin resistance. Moreover, metformin targets the respiratory complex I of the electron transport chain in the mitochondria of preneoplastic and neoplastic cells, reducing energy consumption in the cell. Other mechanisms on which Metformin play an action are: mammalian target of rapamycin (mTOR, crucial to tumor cell metabolism), adenosine mono-phosphate-activated protein kinase (AMPK), mitochondrial glycerophosphate dehydrogenase (mGPDH), and the nuclear factor kB (NF-kB) (Chan, 2016). Importantly, some studies suggest that metformin could cooperate with chemotherapeutic drugs (Kushchayeva et al., 2014; Yoshida et al., 2020).

Controversially, emerging evidence suggest that metformin has potential clinical applications in stem cell medicine, regenerative medicine and anti-aging (Jiang and Liu, 2020). Indeed, several studies have demonstrated that metformin promotes osteogenic, neuronal, myogenic, and adipogenic differentiation with varying results (Zhan et al., 2020; Ould-Brahim et al., 2018; Dadwal et al., 2015; Ahn and Cho, 2017; Fatt et al., 2015; Smieszek et al., 2018). Metformin induces cell proliferation at low concentrations‘ but it has anti-tumor activity at higher concentrations and can inhibit the proliferation of cancer cells (Nashif et al., 2023). At present, research is limited to *in vitro* and experimental animal models, however it can be hypothesized that the contradictory effects of metformin might be dependent upon specific cell types and/or dose differences.

Results from clinical studies regarding the anti-cancer effects of metformin are rather controversial. Indeed, while some retrospective-case control studies, have shown that metformin is able to reduce overall cancer incidence and mortality by 10%–40% (IDF, 2021), RCTs failed to show a significant reduction in cancer incidence and mortality (Stevens et al., 2012; Wen et al., 2022). This discrepancy could be due, at least in part, to the short follow-up time and/or to the biological heterogeneity of cancer. Given its multiple pathways of action and its anti-cancer effects observed in different types of cancer, metformin is the anti-diabetic drug that was most studied in the field of TC. Here will follow a summary of the crucial studies that highlight the importance of metformin in the treatment of TC.

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Similarly, some authors observed that metformin users had a lower clinical severity of TC at presentation and a longer disease free survival in metastatic patients (Jang et al., 2015), while other authors did not observe any difference in the risk of developing metastases when compared with non-treated patients (Noh et al., 2019). Among the possible mechanisms contributing to the anti-tumoral effect of metformin in TC, a TSH-lowering effect can be observed in patients treated with metformin, for several clinical condition (Isidro et al., 2007; Rotondi et al., 2011; Cappelli et al., 2012; Cappelli et al., 2014). This effect would be specific for TC and coud play a role especially in combination with other therapies. Nevertheless, it must be acknowledged that available data all come from retrospective and non-randomized studies. High quality data coming from prospective randomized trials on the use of metformin, alone or in combination, in the treatment of TC are currently lacking.

Interesting findings emerged also from studies *in vitro* on different types of thyroid cells (human anaplastic, differentiated cancer cells, as well as human normal and cancer thyroid primary cell cultures, TC stem cells and rat follicular thyroid cells). These studies highlighted several anti-cancer effects exerted by metformin on TC cells including: i) induction of cell death, ii) reduction of cell growth, iii) inhibition of the metastatic potential iv) modulation of chemokines in the tumor microenvironment, v) de-differentiating effects (increase in iodine up-take) (Chen et al., 2012; Klubo-Gwiezdzinska et al., 2013; Cho et al., 2014; Moon and Mantzoros, 2014; Rotondi et al., 2015; Kim et al., 2018a; Klubo-Gwiezdzinska et al., 2012; Han et al., 2015; Kheder et al., 2017; Nozhat et al., 2018; Ye et al.; Sloot et al., 2019; Durai et al., 2021; Morale et al., 2022). The reduction of cell growth and viability showed in TC cells were due to the regulation by metformin of the AMPK-mTOR pathway, inhibiting cell cycle progression and inducing apoptosis (Klubo-Gwiezdzinska et al., 2012; Klubo-Gwiezdzinska et al., 2013; Thakur et al., 2019). Other mechanisms explaining the effects of metformin on thyroid cell growth and viability are the downregulation of cyclin D1, the increase of the ER stress and the reduction of mRNA levels of AKT, PI3K, and FOXO1 LRP2 and p-JNK, genes that play a crucial role in cell proliferation and survival (Oyadomari and Mori, 2004; Bikas et al., 2015; Nozhat et al., 2018; He et al., 2020). Moving to the ability of metformin to reduce the metastatic potential, it was demonstrated that the drug not only reduced the migration of MTC cancer cells (Klubo-Gwiezdzinska et al., 2012), but also modified the expression of several markers of the epithelial-tomesenchimal-transition (EMT) (Han et al., 2015). A further interesting anti cancer effect of metformin was the ability to reduce the TNF-α-induced CXCL8 secretion by thyroid cells *in vitro*. CXCL8 is a protumorgenic chemokine, thus, this CXCL8-lowering effect of metformin was considered as a further indirect anticancer property of the drug. Metformin was also shown to play a potential role in the re-differentiation of TC cells. Indeed, undifferentiated TC cells express low levels of sodium/iodine-symporter (NIS), a protein membrane crucial for iodine uptake by thyrocites and consequently essential for the efficacy of RAI, the most effective therapy for TC in case of not complete surgical removal. A recent study in ATC cells showed that metformin increased NIS mRNA and protein expression (as well as mRNA of thyroglobulin, TSHR, and NKX2.1) acting also as a demethylating agent (Durai et al., 2021).

Finally, some studies also showed that metformin could synergize with other drugs increasing their anti-cancer effects. Indeed metformin showed synergic effects with Sorafenib (a multikinase inhibitor) (Chen et al., 2015), with vemurafenib (a selective inhibitor of BRAFV600E), with gemigliptin (dipeptidyl peptidase-IV inhibitor) (Kim et al., 2018a) and pioglitazone (a TDZ) (Kim et al., 2018a; Ozdemir Kutbay et al., 2020) in several TC models (Hanly et al., 2015; Durai et al., 2021).

These *in vitro* studies make metformin desirable for potential repositioning as anti-cancer compounds in the treatment of TC, thus several studies are still ongoing to deeply characterize its effect.

Phenformin

In the late 1950s, Phenformin was introduced in the United States for the treatment of non-insulin-dependent (NIDDM) and was 50 times more powerful than metformin. Despite this, it was removed from the market in the late 1970s because of its high risk of lactic acidosis (Luft et al., 1978). The higher efficacy of phenformin compared with metformin seems to be due to different modes of entry into cells. Indeed, the fact that administration of phenformin can induce lactic acidosis, while metformin does not, suggests that these two biguanides act through different pathways (García Rubiño et al., 2019).

Various *in vitro* and *in vivo* studies performed in different types of tumors, highlighted the ability of phenformin to reduce cancer cell proliferation strongly than metformin (Janzer et al., 2014).

Up to now, only one study demonstrated the ability of phenformin to reduce cell viability in TC cells. Moreover, phenformin, at non-cytotoxic concentrations, had also an indirect anti-cancer effect through modulation of the chemokine milieu within the thyroid tumor microenvironment (inhibition of CXCL8 secretion) (Rotondi et al., 2018). These data expand the potential benefits of this molecule as an antitumor drug *in vivo* (Rotondi et al., 2015). Furthermore, phenformin could also display synergism with other anti-cancer molecules (i.e., immune-therapeutics, chemotherapeutic).

In 2011, a study revealed that the administration of phenformin in combination with 2-deoxyglucose can prevent the development of lactic acidosis, reducing toxicity. Therefore, in terms of cancer therapy, perhaps phenformin should be reevaluated (Lea et al., 2011).

As far as thyroid cancer is concerned, *in vitro* and *in vivo* studies focused on the potential anti-cancer effects of metformin and phenformin. A parallel of the effect reported for two biguanides (metformin and phenformin) in thyroid cancer and other solid cancer should be overviewed. Besides the well-known targets such as the inhibition of the mTOR signaling pathway, several additional metformin and phenformin targets (e.g., mGPDH, ATF3, STAT3, GSK3, cyclins) have been identified in other cancers (Thakur et al., 2019). Induction of cell cycle arrest, reduction of viability and induction of ROS were observed in several types of cancers by both drugs. Thus, the anti-cancer effects of both compounds appear to be exerted through several modes of action (action on AMPK, cell cycle, mytocondrial complex-I), likely occurring without a cancer type specificity.

On the other hand, other biguanides (not limiting to anti-diabetic compounds) were tested for their potential anti-cancer properties in different types of cancer. Just to give few examples, buformin and phenformin were showed to inhibit the viability of pituitary cancer cells *in vitro* (Vázquez-Borrego et al., 2019). Chemical modification of metformin into sulfenamides and sulfonamides has also improved the cellular accumulation of these compounds in cancer cells, with a subsequent increase in their cytotoxic efficacy. Many sulfonamide derivatives of metformin exerted cytotoxicity in human breast cancer cells (MCF-7 or MDA-MB-231, or both) induced particularly by methylated phenyl sulfonamides and was associated with their ability to arrest the cell cycle in the G0/G1 phase and subsequently to cause apoptosis (Torunoglu et al., 2023). The novel biguanide MC001 showed much stronger antitumor effect and relatively weaker proglycolytic activity compared with metformin as shown *in vitro* in colorectal cancer cells (Fu et al., 2022). In breast cancer cell lines, it was shown that Cycloguanil and its most promising analogue, NSC127159, were shown to inhibit Dihydrofolate reductase (DHFR), an established anti-cancer drug target whose inhibition disrupts folate metabolism and STAT3-dependent gene expression. A very recent study demonstrated that novel biguanide derivative, IM176, induces prostate cancer cell death *in vitro* by modulating the AMPK-mTOR and androgen receptor signaling pathways (Kim et al., 2023). A study on glioblastoma stem cells reported that phenformin, moroxydine, proguanil and cycloguanil exerted a significant impairment of glioblastoma stem cells proliferation, invasiveness and self-renewal (Barbieri et al., 2018). In 2015, Wysham et al., performed a comparative study of metformin and the novel biguanide NT1014 demonstrating anti-proliferative activity of metformin and NT1014 in ovarian cancer cell lines by inducing cell cycle arrest in G1 phase followed by apoptosis. *In vivo*, NT1014 reduced tumor weight by 61%, whereas metformin by only 32%. A study on bladder cancer showed that proguanil induces autophagic death of BC cells by specific binding to EGFR and inhibiting its expression.

Taken together the anti-cancer effect of biguanides is being studied in several types of cancer suggesting the potential repurpositioning not only of the anti-diabetics, but also of numerous other biguanides-related compounds.

Sodium-glucose transporters (SGLT)-inhibitors

The treatment of T2DM with glyflozines, sodium-glucose co-transporter 2 inhibitors, represents a new therapeutic approach (Devineni and Polidori, 2015). These classes of inhibitors act by decreasing renal glucose uptake and increasing urinary glucose excretion. SGLT2 is located in the initial segment of the proximal tubule, and is responsible for 80%–90% of reabsorption, while SGLT1s reabsorb the remaining 10%–20% (DeFronzo et al., 2017).

Among SGLT2 inhibitors, three of them have been approved by the FDA and EMA: canagliflozin (2013), dapagliflozin (2014) and empagliflozin (2014); three other compounds have been approved in Japan (Ipragliflozin, Tofogliflozin, Luseogliflozin), while others are in development (Ertugliflozin and Sotagliflozin) (Devineni and Polidori, 2015). Recent studies have highlighted that SGLT2 inhibitors, including canagliflozin and Dapagliflozin, can inhibit cancer and colorectal cell growth through inhibition of SGLT2-mediated glucose uptake (Dutka et al., 2022).

Canagliflozin

Canagliflozin [(1 *S*)-1,5-andro-1-[3-[[5-(4-fluorophenyl)-2-tienil]metil]-4-metilfenil]-d- GLUCITOLO emirate], is a C-glycosyl compound that is used (in the hemihydrate form) for the treatment of T2DM through inhibition of sodium/glucose co-transporter 2 (Choi, 2016) and was approved by the FDA in 2013. Canagliflozin is an orally active selective SGLT2 inhibitor. It is administrated orally and is rapidly absorbed, reaching peak plasma concentration in 1–2 h (Devineni and Polidori, 2015). Canagliflozin acts both delaying intestinal glucose absorption as well as increasing urinary glucose excretion; this mechanism contributes to lower postprandial blood glucose.

It was initially approved by the FDA in 2013 for the management of T2DM and later approved in 2018 for a second indication of reducing the risk of cardiovascular events in patients diagnosed with T2DM. Recently, it was observed that canagliflozin promotes AMPK activity by inhibiting mitochondrial respiration in embryonic kidney cells and mouse cells (Hawley et al., 2016). As with biguanides, canagliflozin inhibits mitochondrial respiration and cell proliferation, suggesting that it may be useful in cancer prevention and treatment.

Studies *in vitro* and *in vivo* have demonstrated the inhibitor effect of canagliflozin on TC cells. The SGLT2 inhibitor could suppress the glycolysis level of TC cells and also interfere with glucose uptake and glycolysis in TC cells. In addition, the treatment with canagliflozin induced cell apoptosis of TC cells (Wang et al., 2022). Furthermore, this anti-diabetic drug increases the activation of ATM/CHK2 in TC cells, indicating DNA damage repair is initiated. In addition, recent studies have shown that Canagliflozin is also able to activate AMPK, through inhibition of complex I of the respiratory chain. This suggests that some therapeutic benefits of canagliflozin could result from the activation of AMPK, rather than inhibition of SGLT2 (Hawley et al., 2016). Thus, further studies are needed to evaluate Canagliflozin as a candidate for repositioning as an anticancer agent, including TC.

DPP-4 inhibitors

These compounds, by preventing deactivation by dipeptidyl peptidase-4 (DPP4) inhibitors, improve the concentration of endogenous incretins (Ahrén et al., 2002; Vangoitsenhoven et al., 2012). Currently, DPP-IV inhibitors are widely used as monotherapy or combination therapy for the treatment of patients with T2DM.

DPP-IV modulates diverse cellular processes including survival, proliferation, and differentiation, and thereby enhances or diminishes tumorigenesis depending on the types or phases of tumors (Havre et al., 2008). In this regard, DPP-IV can be either overexpressed or underexpressed in human solid tumor tissues,

suggesting the possible role of DPP-IV as a potential diagnostic marker and therapeutic target in solid tumors (Havre et al., 2008).

Initially, a register-based study from Taiwan suggested that the administration of DPP-4 inhibitors could be associated with an increased risk of TC (Tseng, 2016). On the other hand, the investigation of DPP-4 inhibitors as potential anti-cancer agents was not discouraged by this study because DPP4 expression in TC was demonstrated to be associated with cellular invasion, promoting TC cell metastasis, and a more aggressive disease in papillary TC (Lee et al., 2017; He et al., 2022). More interestingly a recent *in vitro* study showed that DPP4 gene silencing inhibits papillary TC cell proliferation and EMT and promotes cell apoptosis (Hu et al., 2021). In addition, in contrast with previous findings, a systematic review and metanalysis by Overbeek et al. (2018), showed that it is not possible to conclude whether DPP-4 inhibitors were associated with an increased risk of site-specific cancer including TC. Another population-based cohort study of patients with T2DM with a concomitant cancer showed that no increased risk of metastasis was associated with DPP-4 inhibitor therapy (Noh et al., 2019). Thus, the targeting of DPP4 was considered as a potential therapeutic strategy for DPP4-expressing TC and further studies were encouraged.

Sitagliptin

Sitagliptin is an oral dipeptidyl peptidase-4 (DPP-4) inhibitor used in conjunction with diet and exercise to improve glycemic control in patients with T2DM. The effect of this medication leads to glucose-dependent increases in insulin and decreases in glucagon to improve control of blood sugar. Sitagliptin was granted FDA approval on 16 October 2006 (National Center for Biotechnology Information, 2023f).

Tseng (2016) showed that among Taiwanese patients with T2DM, sitagliptin use may be associated with an increased risk of TC. On the other hand, this compound showed a reduction of TC cell viability, proliferation, and some aspects related to the metastatic process *in vitro*. Indeed, Hu et al. (2021) reported cytotoxic effects of Sitagliptin on TC cell lines TPC-1 and GLAG-66 as well as a reduction of cell proliferation in both TC cell lines. Interestingly, Sitagliptin was able to reduce TC cell migration by influencing the expression of some markers of the epithelial-to-mesenchimal-transition (EMT) which ultimately drives cancer cell migration (Hu et al., 2021). In addition, a recent *in silico* molecular docking study regarding the *DPP4/CTNNB1/MET* signatures showed that stagliptin could be a potential TC drug, however more investigations are surely needed to confirm it (Cheng et al., 2022).

Gemigliptin

Gemigliptin is an orally bioavailable inhibitor of DPP-4, with hypoglycemic and potential renoprotective activities. Upon administration, gemigliptin binds to DPP-4 and inhibits the breakdown of the incretin hormones, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP). This prolongs incretin activity, increases postprandial insulin secretion from pancreatic beta cells, decreases glucagon secretion, delays gastric emptying, and lowers blood glucose levels (National Center for Biotechnology Information, 2023a).

In 2017, Kim et al. (2017) demonstrated that gemigliptin was able to induce TC cell death *in vitro*. Another study of the same group further demonstrated the cytotoxic *in vitro* effect of gemigliptin in TC cells also showing an increase in its cytotoxic activity when combined with one histone deacetylase inhibitor (PXD101) (Kim et al., 2018b). More interestingly, these investigators showed that gemtigliptin in combination with another anti-diabetic compound, but belonging to a different class (biguanides), Metformin (Kim et al., 2018a), exerts a stronger adverse effect on TC cells *in vitro*. Indeed, TC cells treated with both gemigliptin and metformin showed synergistic cytotoxicity of two agents, exerted by acting on Akt and AMPK pathways. The same study also showed that gemigliptin increased the inhibition of cell proliferation and migration induced by metformin by involving of ERK,MMP-2-9, p53, p21, VCAM-1, and (Kim et al., 2018a).

Thiazolidinediones

Thiazolidinediones (TZDs), (also called “glitazones”) were introduced in 1996 for T2DM, when troglitazone (Rezulin; Parke-Davis/Warner-Lambert) was approved by the Food and Drug Administration. TZDs uniquely target insulin resistance, which is a core physiologic defect in T2D, and significantly improve glucose control. Unfortunately, due to severe hepatic and cardiovascular side-effects, most TZDs were removed from the clinical use, with on pioglitazone still recommended by most guidelis as an anti-diabetic drug. However, TZDs improve insulin action in adipose, hepatic tissue and muscle, agonizing with of peroxisome proliferator–activated receptor-γ (PPAR-γ) nuclear receptors. PPAR-γ activation is translated into different vascular and metabolic effects including the upregulation and downregulation of different genes essential for e lipid and glucose metabolism, but also for inflammatory response. *In vitro* data highligted that PPAR*γ* could be suggested as targets for TC therapy (Chung et al., 2002; Martelli et al., 2002; Hayashi et al., 2004).

Troglitazone

Troglitazone was the first TZD approved for use in the United States and was licensed for use in T2DM in 1997, but withdrawn 3 years later because of the frequency of liver injury, including acute liver failure, associated with its use. Troglitazone has several recognized therapeutic properties as a hypoglycemic agent, an antioxidant, a vasodilator agent, an anticonvulsant, an anticoagulant, a platelet aggregation inhibitor, an antineoplastic agent, an EC 6.2.1.3 (long-chain-fatty-acid--CoA ligase) inhibitor and a ferroptosis inhibitor (National Center for Biotechnology Information, 2023h).

A first *in vitro* evaluation of the potential anti-cancer effects of troglitazone showed that the compound was efficient in inducing re-differentiation of TC cells *in vitro*, enhancing RAI-uptake. Subsequent studies showed that troglitazone inhibited anaplastic TC cell proliferation *in vitro* and increased the effect of paclitaxel (Copland et al.).

Combined treatment with ovastatin and lovastatin (a cholesterol-lowering agent) inhibited epidermal growth factor-induced migration

of anaplastic TC cells (Chin et al., 2017). Moreover, an *in vitro* and *in vivo* mouse model of anaplastic TC showed that troglitazone + Lovastatin display anti-cancer effects such as reduction of cell proliferation and tumor regression (Zhong et al., 2018).

Pioglitazone

Pioglitazone is both a PPARα and PPARγ agonist with hypoglycemic activity and an insulin-sensitizing role.Pioglitazone is the only drug of the TZD class still commonly used in clinical practice. Moreover, it is recognized to be a pantothenate kinase inhibitor, a long-chain-fatty-acid--CoA ligase inhibitor, a ferroptosis inhibitor, a cardioprotective agent, an antidepressant, and a geroprotector (National Center for Biotechnology Information, 2023g).

Several studies reported that pioglitazone could exert benefits against TC.

Indeed, it was shown that Pioglitazone increased the iodide uptake *in vitro* by thyroid cells (Fröhlich et al., 2005) and exerted a reduction of cell proliferation in anaplastic TC cells *in vitro* (Antonelli et al., 2009). Pioglitazone was demonstrated to induce cellular lipid accumulation in TC cells *in vitro*. Moreover, it was demonstrated that TF-1 interacts with PPFP to inhibit the pro-adipogenic response to pioglitazone and that the ability of pioglitazone to decrease TTF-1 expression contributes to its pro-adipogenic action (Xu et al., 2016). Ozdemir Kutbay et al. (2020) showed that the combination of metformin and pioglitazone induced significant reductions in the level of oncogenic genes (AKT3, DEPTOR, EIF4E, ILK, MTOR, PIK3C, and PRKCA) in TC cells. This finding would indicate that TC progression could be prevented and these genes could be selected as therapeutic targets (Ozdemir Kutbay et al., 2020).

Interesting data regarding the potential anti-cancer activity of pioglitazone come from studies performed with a transgenic mouse model characterized by a PAX8-PPARγ fusion protein (PPFP) (found in 30% of follicular thyroid carcinomas). This particular fusion confers oncogenic capacity in transgenic mice. A 2011 *in vivo* study demonstrated that, in this mouse model, the administration of Pioglitazone induces a proadipogenic antitumor response, with the final result of preventing metastasis and reducing tumor size (Dobson et al., 2011). Another 2017 study in the same mouse model showed that pioglitazone exerted the induction of infiltration of immune cells (macrophages and T cells) only in the presence of PPFP (Zhang et al., 2017) highlighting the importance of the use of this compound in that specific clinical setting. Indeed, a subsequent clinical trial showed that pioglitazone may be therapeutic in patients with TC bearing PPFP (Giordano et al., 2018). Among the available clinical data in human subjects, in 2012 one case report showed that pioglitazone treatment could have some positive effects in radioiodine-negative and progressive DTC patients (Rosenbaum-Krumme et al., 2012). Moreover, Tseng (2014a) showed a null association between pioglitazone use and TC risk in patients with T2DM.

Finally, a comprehensive study on diagnosis, prognosis, and potential drug screening for papillary thyroid carcinoma (PTC), based on five hub lncRNAs, identified pioglitazone among the potential drugs that could be effective for TC treatment (Li et al., 2021).

Rosiglitazone

Rosiglitazone was marketed both alone (Avandia) (National Center for Biotechnology Information, 2023b) and combined with metformin (National Center for Biotechnology Information, 2023e) (Avandamet) or with glimepiride (National Center for Biotechnology Information, 2023d) (Avandaryl). Like other TZDs, activates PPARs and is a selective ligand of PPARγ with no PPARα-binding action. Rosiglitazone display well known effect on insulin resistance, but also shows anti-inflammatory effects (Lombardi et al., 2008).

Several studies suggest that rosiglitazone could have an anti-cancer effect on TC. Rosiglitazone inhibited anaplastic TC cell proliferation *in vitro* and increased the effect of the chemotherapy paclitaxel (Copland et al.). In the study by Aiello et al. (2006) performed *in vitro* on anaplastic thyroid cells, it was demonstrated that the treatment with rosiglitazone reduced anchorage-dependent and -independent growth and migration of TC cells, and increased apoptosis rate by reducing Bcl-X(L) expression and caspase-3 and -7 activation. The effect of rosiglitazone on cellular growth was associated with cell cycle arrest and with an increase of cyclin-dependent kinase inhibitors p21 (cip1) and cyclin-dependent kinase regulator p27 (kip1), a decrease of cyclin D1, and inactivation of Rb protein. Finally, rosiglitazone increased the expression of thyroid-specific differentiation markers (Aiello et al., 2006). In an *in vitro* study, under normoxic or hypoxic conditions, it was reported that rosiglitazone inhibited TC cell growth and increased NIS protein expression. This data is of further support the ability of rosiglitazone to induce re-differentiatio of TC cell (Chen et al., 2020). Finally, a recent study showed that rosiglitazone significantly inhibited transforming growth factor-beta1 (TGF-β1)-induced EMT-associated processes such as fibroblast-like morphological changes, EMT-related protein expression, and increased cell migration and invasion in BCPAP and K1 TC cells. Furthermore, rosiglitazone suppressed TGF-β1-induced MMP-2 expression and phosphorylation of p38 MAPK, but not ERK1/2 (Jin et al., 2021).

The possible role of rosiglitazone as anti-cancer agent in TC was also investigated in the clinical setting. Philips et al. showed an increase in RAI-uptake upon treatment with rosiglitazone (Philips et al., 2004). The successful induction of RAI uptake (decreased thyroglobulin levels and decreased in tumor size) after treatment with rosiglitazone was showed in two studies performed in metastatic DTC patients (Elias and Lizotte, 2006; Elola et al., 2011). In addition an increased RAI-uptake in therapeutic ^{131}I scans (Kebebew et al., 2006; Tepmongkol et al., 2008) was reported in a phase II clinical trial that on the other hand concluded a not complete response of patients (Eisenhauer et al., 2009; Kebebew et al., 2009). It should be acknowledged that these studies had several limitations, including the limited accuracy of the technique of 131I scans and the unknown status of receptor expression of the treated TC. The status of a currently ongoing trial (NCT 00098852) with rosiglitazone is not known.

In 2013 Tseng et al. by using the National Health Insurance (NHI) reimbursement databases of Taiwan showed that rosiglitazone use may reduce the risk of TC in patients with T2DM (Tseng, 2013).

Ciglitazone

Ciglitazone born in 1980 and is considered to be the prototypical of TZDs, indeed was never used as a medication. Several analogs were later developed, including pioglitazone and troglitazone. Ciglitazone also exerts anti-inflammatory activity through the modulation of nuclear factor-kappaB-mediated pathways. In addition, this agent inhibits angiogenesis by reducing vascular endothelial growth factor (VEGF) production and inhibits the growth of melanoma cells by inhibiting the expression of (C-X-C motif) ligand 1 (CXCL1) (National Center for Biotechnology Information, 2023c).

In the *in vitro* study by Martelli et al. (2002), it was demonstrated that the treatment with ciglitazone inhibited the growth of several types of thyroid carcinoma cell lines *in vitro* in a time-dependent manner. Moving to anaplastic TC, an *in vitro* study demonstrated that in a panel of six anaplastic thyroid cancer (ATC) cell lines, the treatment with ciglitazone reduced anchorage-dependent and -independent growth and migration, and increased the apoptosis rate of TC cells (Aiello et al., 2006). Another *in vitro* study showed that ciglitazone induced apoptosis of TC cells by affecting the cytochrome-c caspase 3 and PTEN-Akt pathways, in addition the necrosis was obtained by affecting the PARP pathway (Chen et al., 2006).

Lobeglitazone

Lobeglitazone activates PPAR-γ and promotes the binding of insulin at fat cells, reduces blood sugar levels, lowers hemoglobin A1C (HbA1C) levels, and improves lipid and liver profiles (National Center for Biotechnology Information, 2023i).

Only one study showed that TC cell lines treated with lobeglitazone *in vitro* showed a significant reduction of TGF-β1-induced EMT-associated processes and EMT markers expression reducing also cell migration and invasion. Moreover, the treatment with lobeglitazone restored TGF-β1-induced loss of E-cadherin, as observed using immunocytochemistry, and suppressed TGF-β1-induced MMP-2 expression and phosphorylation of p38 MAPK, but not ERK1/2 (Jin et al., 2021).

Anti-diabetic drugs repositioning for tumor treatment: the other face of the coin

The previous chapter is suggestive of a collective practicable repositioning of most of the anti-diabetic drugs, in particular for the treatment of TC. On the other hand, not all anti-diabetic drugs are free from potential side effects, which makes them not suitable for repositioning therapy of TC. In particular, a specific class, GLP-1 analogs deserves to be discussed given their potential pro-tumorigenic effects in TC.

GLP-1 analogues

Glucagon-like peptide-1 (GLP-1) receptor agonists are effective treatments for T2DM which lower glucose concentrations without weight gain (often with weight loss) and with low risk for hypoglycemia (Hinnen, 2017).

GLP-1-based therapies represent a significant advance in the treatment of T2D. One of these medications is liraglutide. FDA in 2014 approved the higher dose version of this compound (known as Saxenda) for chronic weight-management treatment. Oher GLP-1 like lixsenatide (Sanofi Aventis, trade name Lyxumia) and albiglutide (GlaxoSmithKline, trade names Epezan and Tanseum) are currently approved or are under consideration for diabetes treatment (Ladenheim, 2015). About the prescription of exenatide and liraglutide, both compounds are contraindicated in MTC patients or multiple endocrine neoplasia syndrome 2 (MEN 2) patients due to the increased incidence of C-cell hyperplasia and tumors combined with elevated calcitonin levels in preclinical studies in rodents. However, these observations have not been replicated in nonhuman primates or humans and are believed to be a rodent phenomenon due to the higher density of GLP-1R on rodent C cells, so the responses obtained on rodents may not be relevant to primates (Bjerre Knudsen et al., 2010; Samson and Garber, 2013; Drab, 2016).

On the other hand, no case report describing medullary thyroid carcinoma has been published in a patient being treated with a GLP-1 receptor agonist who had a morphologically normal thyroid and low calcitonin concentrations before such treatment. Efficient surveillance of an extremely large number of patients would be required to confirm or reject such a report (Nauck, 2013). Regarding this class of molecules, studies in rats have led to mixed results. Some studies conducted in rodents, with exanatide and liraglutide, showed an association regarding the development of thyroid C-cell tumors after long exposures to overtherapeutic doses (Bjerre Knudsen et al., 2010). Liraglutide-induced C-cell hyperplasia and C-cell adenomas in mice and rats, and was also associated with a significant increase in C-cell carcinomas in rats and female mice administered the highest liraglutide dose tested (Aroda and Ratner, 2011). Studies with exenatide showed an increase in the incidence of C-cell adenomas in rats (female), exposed to 130-fold higher than the clinical dose of exenatide. Of note, no effect on C-cell was observed in mice similarly treated (Bethel et al., 2019). In contrast to results obtained in rodents, *in vivo* studies in cynomolgus monkeys administering liraglutide showed no effect on the relative fraction of C cells in the thyroid gland after 87 weeks. The risk of TC associated with liraglutide has been examined in rodent and non-human primate animal model studies. Indeed the liraglutide long-term treatment has been associated with thyroid C-cell hyperplasia and tumors in rodents, but not in monkeys.

Semaglutide has received an official box warning for thyroid C-cell tumors in the United States. This caution is based only on data from rodent studies and is not unique for semaglutide amongst the GLP-1RA (Bjerre Knudsen et al., 2010; Pyke and Knudsen, 2013). In contrast to liraglutide (Bjerre Knudsen et al., 2010) and lixisenatide (European Medicines Agency, 2012) in rats, the drug dulaglutide did not show an increase in thyroid C-cell tumors in rats. However dulaglutide doses greater than 0.5 mg/kg were demonstrated to increase hyperplasia of thyroid C cells (Byrd et al., 2015).

These data suggest that rodents are particularly sensitive to the effects of GLP-1 agonists on thyroid C cells but these foindings could not be considered as predictive of an increased risk of thyroid C-cell tumors in patients under GLP-1 receptor agonist therapy (Byrd et al., 2015).

Insulin

Insulin is a widely prescribed glucose-lowering agent (Sims et al., 2021) especially in patients affected by type 1 diabetes mellitus (T1DM) as well as in some patients with Type 2 diabetes mellitus. The potential cancerogenic property of insulin is among the safety concerns related with long-term insulin therapy. Indeed, insulin is a growth factor, and the administration of exogenous insulin could, at least theoretically, stimulate tumour growth (Karlstad et al., 2013). The oncogenic effect of insulin could be due to the overexpression of insulin receptor by cancer cells, but also to its ability to interact with the IGF1 receptor, especially at supraphysiologic doses (Baricevic et al., 2015; Gallo et al., 2018) and with the use of long-acting analogues (Sciacca et al., 2010). Indeed, several studies have demonstrated that aberrant IGF signaling plays a critical role in the pathogenesis and progression of several types of cancer, including lung, breast, colon, prostate, ovary, pancreas, and thyroid (Bowers et al., 2015).

Nevertheless, data coming from observational studies are still conflicting and inconclusive, since some authors observe an association between insulin therapy and increased cancer risk (Currie et al., 2009; Tseng, 2019; Vicentini et al., 2022), while others failed to register any association (Pocock and Smeeth, 2009; But et al., 2017; Tan et al., 2017).

The possible effect of insulin therapy as a risk factor for thyroid cancer comes from pre-clinical data suggesting that insulin signaling is a key mediator in thyroid cancer cell growth. Indeed, early *in vitro* studies on rat thyroid follicular cells showed that concurrent treatment with insulin and TSH significantly increased the cell number compared to treatment with TSH alone (Tramontano et al., 1986).

Moreover the IGF1 axis, which can be stimulated by excessive levels of circulating insulin, is one of the key pathways involved in proliferative responses in both normal and neoplastic thyroid cells (Vella et al., 2001; Malaguarnera et al., 2012; Vella and Malaguarnera, 2018; Manzella et al., 2019). Thyroid cancer cells also over-express the insulin receptor (IR), especially isoform IR-A. IR/IGF-1 receptor hybrids and IR-A lead to an over-activation of the IGF pathway, causing an enhanced mitogenic signaling and cancer development (Malaguarnera et al., 2011).

Only few clinical studies up to now evaluated the relationship between insulin therapy and thyroid cancer risk (Kushchayeva et al., 2022). A 2014 studies based on data from the reimbursement databases of all Taiwanese diabetic patients from 1996 to 2009 evaluated the incidence of thyroid cancer according to the use, duration and dosage of therapy with human insulin. The results failed to show any significant association between human insulin use and risk of developing thyroid cancer, even at higher doses (Tseng, 2014b). Similarly, the study by Luo et al. (2016), did not show any association between insulin use and incidence of thyroid cancer.

Most clinical data on insulin signaling in thyroid cancer derive from the hypothesis that insulin-resistance, typical of obesity, metabolic syndrome and T2DM, could be a risk factor for thyroid cancer development (Malaguarnera et al., 2017). The only evidence of a possible positive correlation between insulin use and thyroid cancer comes from a 2011 study analyzing data from the Danish National Diabetes Register and Cancer Registry. The aim of the study was to evaluate if diabetes status, duration of diabetes and insulin use could be risk factors for the development of several types of cancer. The results showed that cancer incidence was higher among diabetic patients using insulin versus non-users. When specifically evaluating thyroid cancer, a significant difference between insulin users versus non-users was observed only in female patients. The risk also increased in relation to disease and therapy duration (Carstensen et al., 2012).

In conclusion, although pre-clinical data would support a role of insulin therapy as a risk factor for thyroid cancer, clinical data are still inconclusive.

Insulin secretagogues

The primary action of secretagogues is to increase the release of insulin by inhibiting ATP-sensitive potassium channels in the pancreatic β-cell membrane. These compounds are classified as sulfonylureas or non-sulfonylureas (glinides) (Davies, 2002). The sulfonylureas have been extensively used to treat type 2 diabetes for nearly 50 years, representing the second and most used oral hypoglycemic drugs after metformin. A first-generation including Tolbutamide, Acetohexamide, Carbutamide, Chlopropamide, and Tolazamide, was introduced in Germany since the 1950s. In the 1980s more potent second-generation sulfonylureas became available (glibenclamide, glibornuride, gliclazide, glipizide, and gliquidone). Lastly, glimepiride, a third-generation sulfonylurea, was introduced in 1995 in the United States (Tan and Nelson, 1996).

Glinides are insulin secretagogues that lack the sulfamide group of the sulfonylureas and differ from sulfonylureas in receptor affinity, binding sites, duration of action and mechanism of absorption and elimination (Culy and Jarvis, 2001). Three glinides have been approved for use: repaglinide, nateglinide, and mitiglinide.

According to data from several meta-analyses, an overall increased cancer risk was reported in patient using sulfonylureas compared with those treated with metformin or other diabetes medications (Wu et al., 2015; Sacks et al., 2018; Mekuria et al., 2019; Chen Y. et al., 2023b). A meta-analysis of 8 studies (3 cohort studies, 3 case-control studies and 2 clinical trials) failed to demonstrate any association between glinides and risk of cancer (Wu et al., 2015).

As concern TC, a recent study by Tseng et al. suggest that among the anti-diabetic agents, only sulfonylurea, and not insulin, was significantly associated with higher risk of TC (Tse and ng, 2012). Hyperinsulinemia or insulin resistance alone might not be responsible for thyroid cell proliferation in patients with type 2 diabetes. A possible explanation could be related to the different effects of insulin on the thyroid gland. Insulin may increase thyroid hormone transcriptional action and reduce TSH level probably through the effect of hypoglycemia on pituitary-thyroid secretory activity (Hu et al., 1994; Schultes et al., 2002). On the other hand, first-generation sulfonylureas have been well known to exert anti-thyroidal effects and may be goitrogenic in animals or human (Hershman and Konerding, 1968; NIKKILA et al., 1960). It is possible that higher level of TSH, even within the normal range, may increase the risk of TC (Kim et al., 2013).

Conclusion

The present review encompassed a roundup of studies on the anti-cancer effects of several anti-diabetic compounds in TC. Some of these compounds not only directly affect TC cells by reducing their viability, proliferation, or their ability to migrate to the metastatic side, but also indirectly affect cancer progression by regulating the secretion of pro-tumorigenic chemokines in the TC microenvironment. The reduction of pro-tumorigenic chemokines within and surrounding the thyroid tumor microenvironment is of benefit for counteracting cancer progression. Among the here reported anti-diabetic compounds, it looks like metformin, given its numerous anti-cancer effects which include reduction of cell growth, promotion of cell death, and reduction of cell migration as well as modulation of TC microenvironment component could be of interest for a potential repositioning. Indeed, metformin shows few side effects and encouraging data observed *in vitro* and *in vivo* on TC models. In addition to metformin, other anti-diabetic drugs belonging to other classes, like TZDs and DPP-4 inhibitors showed encouraging results. In this view, it would be of interest to investigate the potential combinatory anti-cancer effects of these compounds. It is important to highlight that not all anti-diabetic drugs appear suitable for repositioning given their potential pro-tumorigenic effects.The effects of anti-diabetica drugs in thyroid cancer are summarized in Figure 1. The example of GLP-1 and medullary TC, although not definitely proven in humans, highlights that anti-diabetic drug repositioning needs to be evaluated specifically for each molecule.

In this view, trials aimed at testing the potential repositioning of these compounds should be designed by also taking into account the mechanism of action of single drugs and potential combination with other drugs or molecules.

Author contributions

AG: Conceptualization, Investigation, Writing–original draft, Writing–review and editing. FC: Conceptualization, Writing–original draft, Writing–review and editing. LC: Writing–review and editing. FM: Writing–review and editing. MT: Writing–review and editing. MR: Conceptualization, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was partially supported by the "Ricerca Corrente."

Acknowledgments

Ministry of Health Italy Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3—Call for proposals No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union—NextGenerationEU; Award Number: Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods—Research and innovation network on food and nutrition Sustainability, Safety and Security—Working ON Foods."

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 19 January 2024
DOI 10.3389/fphar.2024.1324001

OPEN ACCESS

EDITED BY
Oriana Awwad,
The University of Jordan, Jordan

REVIEWED BY
Kusmardi Kusmardi,
University of Indonesia, Indonesia
Mohammad Alwahsh,
Al-Zaytoonah University of Jordan, Jordan

*CORRESPONDENCE
Yasser Bustanji,
ybustanji@sharjah.ac.ae

RECEIVED 18 October 2023
ACCEPTED 04 January 2024
PUBLISHED 19 January 2024

CITATION
Dakilah I, Harb A, Abu-Gharbieh E, El-Huneidi W, Taneera J, Hamoudi R, Semreen MH and Bustanji Y (2024), Potential of CDC25 phosphatases in cancer research and treatment: key to precision medicine. *Front. Pharmacol.* 15:1324001. doi: 10.3389/fphar.2024.1324001

Potential of CDC25 phosphatases in cancer research and treatment: key to precision medicine

Ibraheem Dakilah[1], Amani Harb[2], Eman Abu-Gharbieh[1,3], Waseem El-Huneidi[1,3], Jalal Taneera[1,3], Rifat Hamoudi[1,3,4], Mohammed H. Semreen[5] and Yasser Bustanji[1,3,6]*

[1]Research Institute of Medical and Health Sciences, University of Sharjah, Sharjah, United Arab Emirates, [2]Department of Basic Sciences, Faculty of Arts and Sciences, Al-Ahliyya Amman University, Amman, Jordan, [3]College of Medicine, University of Sharjah, Sharjah, United Arab Emirates, [4]Division of Surgery and Interventional Science, University College London, London, United Kingdom, [5]College of Pharmacy, University of Sharjah, Sharjah, United Arab Emirates, [6]School of Pharmacy, The University of Jordan, Amman, Jordan

The global burden of cancer continues to rise, underscoring the urgency of developing more effective and precisely targeted therapies. This comprehensive review explores the confluence of precision medicine and CDC25 phosphatases in the context of cancer research. Precision medicine, alternatively referred to as customized medicine, aims to customize medical interventions by taking into account the genetic, genomic, and epigenetic characteristics of individual patients. The identification of particular genetic and molecular drivers driving cancer helps both diagnostic accuracy and treatment selection. Precision medicine utilizes sophisticated technology such as genome sequencing and bioinformatics to elucidate genetic differences that underlie the proliferation of cancer cells, hence facilitating the development of customized therapeutic interventions. CDC25 phosphatases, which play a crucial role in governing the progression of the cell cycle, have garnered significant attention as potential targets for cancer treatment. The dysregulation of CDC25 is a characteristic feature observed in various types of malignancies, hence classifying them as proto-oncogenes. The proteins in question, which operate as phosphatases, play a role in the activation of Cyclin-dependent kinases (CDKs), so promoting the advancement of the cell cycle. CDC25 inhibitors demonstrate potential as therapeutic drugs for cancer treatment by specifically blocking the activity of CDKs and modulating the cell cycle in malignant cells. In brief, precision medicine presents a potentially fruitful option for augmenting cancer research, diagnosis, and treatment, with an emphasis on individualized care predicated upon patients' genetic and molecular profiles. The review highlights the significance of CDC25 phosphatases in the advancement of cancer and identifies them as promising candidates for therapeutic intervention. This statement underscores the significance of doing thorough molecular profiling in order to uncover the complex molecular characteristics of cancer cells.

KEYWORDS

cancer, CDC25, natural compounds, AI (artificial intelligence), precision medicine, omics

1 Introduction

Cancer is the unregulated and rampant replication of cells that leads to the disease one can witness in all population groups. Cancer diagnoses have been steadily rising in the younger demographic worldwide (Ugai et al., 2022) and the search for more effective and targeted therapies continues. Some of the deadliest of these reported globally were breast cancer, stomach cancer, non-melanoma skin cancer, colon and rectum cancer, cancers affecting the respiratory system/tract, and prostate cancer have increased (Lin et al., 2021; Semreen et al., 2022; Ahmed et al., 2023; Hagyousif et al., 2023). Unfortunately, the progress of ready-to-market therapeutics has not increased at the same rate with many of the drugs still in clinical trials due to confounding data, poor clinical trial management and experimentation on top of the inherent duration of sufficient clinical experimentation for such novel treatments (Schilsky, 2010). Novel treatments are sought for their effectiveness against current treatment-resistant and aggressive tumours, increasing the prognosis for cancer patients (Motawi et al., 2014; Alfarouk et al., 2015). Another avenue that has in recent years been making strides in delivering a more personalized and purported more effective treatment has been the field of precision medicine. Precision medicine considers the pharmacological and genomic effects that arise from person to person delivering an effective treatment on a case-by-case basis.

2 Definition and significance of precision medicine

Precision medicine, also known as personalized medicine, is a healthcare technique that incorporates each patient's distinctive characteristics when making decisions regarding their medical treatment. It reflects that people differ in terms of their genetic profile, environmental exposures, lifestyle choices, and illness features. Precision medicine, instead of a one-size-fits-all strategy, tries to personalize medical therapies to each patient's needs (Tsimberidou et al., 2020). Founded on the recognition that individuals may respond differently to therapies depending on the previously mentioned profiles. Precision medicine tries to find particular biomarkers or genetic abnormalities linked with certain diseases, such as cancer, by applying modern technologies involving combinations of genomic sequencing, molecular profiling, and bioinformatics (Olivier et al., 2019). This helps healthcare providers forecast an individual's likelihood of acquiring particular disorders and select the most effective treatment choices. Observations from many cancers cases have shown to be unresponsive to traditional chemotherapy, the reasons which can be found in the patient tumour molecular profile.

Precision medicine is critical in cancer research due to how it improves diagnostic accuracy, optimizes treatment choices, and improves patient outcomes. Precision medicine can uncover specific genetic variations or modifications fuelling the growth of cancer cells by examining a patient's tumour at the molecular level (Roelands et al., 2023). This knowledge can help lead to the development of tailored medicines that directly target these molecular anomalies, boosting the likelihood of a positive response (Tsimberidou et al., 2020). More accurate methods of screening arising from advancements in algorithmic imaging and sorting and learning from data sets have reduced extraneous harm as fewer patients would need to undergo radiotherapy (Avanzo et al., 2020). Furthermore, precision medicine promotes the development of biomarker-driven clinical trials, which aim to test the efficacy of new medicines on patient subgroups with similar genetic or molecular traits. This approach enables researchers to find novel therapeutic targets and hasten the development of innovative medications that are more effective and less hazardous (Abushawish et al., 2022; Ahmed et al., 2023).

3 Introduction to CDC25 phosphatases and their role in cell cycle regulation

These new treatments attempt to tackle the mechanism by which cell replication or innate repair goes awry leading to tumorigenesis. One promising avenue of research is the use of inhibitors of cell cycle division. Cycle Division Cycle 25 (CDC25), a CDK phosphatase (Lavecchia et al., 2009), plays a vital role in regulating the factors of cyclic expression observed for cell cycle progression making it an attractive target for targeted cancer therapeutics. As such, CDC25 phosphatases are some of the more attractive targets for cancer therapy, especially for cancer types that are more aggressive and more difficult to treat such as receptor protein triple-negative breast cancer (He et al., 2013; Liu et al., 2018).

CDC25 proteins are phosphatases expressed during the cell cycle regulating factors including CDKs (Hoffmann and Karsenti, 1994). CDKs are crucial for the progression and regulation of the interphase and entry into mitosis. The regulation of CDKs occurs through the phosphatase action of the three different paralogues of CDC25. The well-documented component of CDC25 isoforms is CDC25A expressed and active during G1/S and G2/M checkpoints, while CDC25B and CDC25C are active during the G2/M checkpoints and have additional roles, including DNA damage repair and regulation of meiosis respectively.

In this review, we will discuss the actions of CDC25 phosphatases in causing neoplastic growth and cell cycle regulation, the overview of CDC25 inhibitors and efficacy in different cancers and the development of CDC25 inhibitors for cancer therapy both *in-vitro* and clinical trials, and finally review the complications and future for human cancer treatments.

4 Exploring the link between precision medicine, CDC25 phosphatases, and the potential of CDC25 inhibitors in cancer treatment

The main targets of precision medicine and *in silico* techniques are the molecular or metabolomic profiles that can be used to accurately predict patients' risk for cancer and detect cancerous tissue at an early stage (Li et al., 2022). Clinical sequencing investigations have established that genomic profiling is feasible in clinical settings and that it is possible to build procedures for informing patients and healthcare professionals about the results of these studies (Forrest et al., 2018). Omics' technique matching scores were associated with better disease control rates, suggesting that

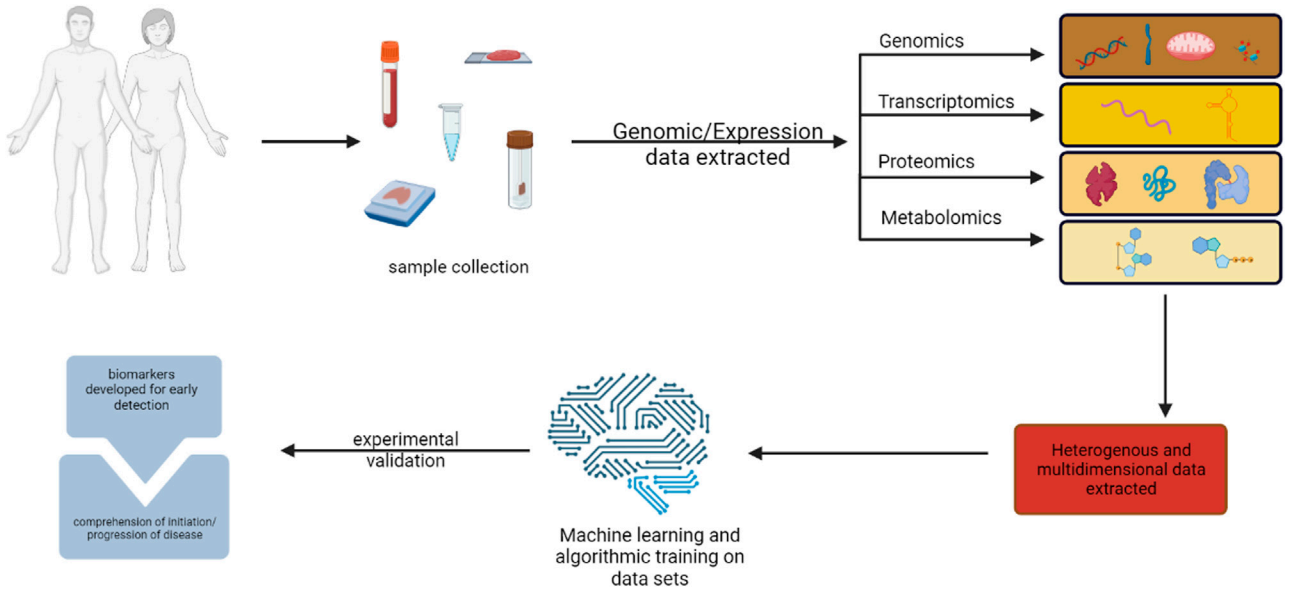

FIGURE 1
illustration of multi-omics methods for finding cancer biomarkers for early diagnosis and pathogenesis. Created with BioRender.com.

customizing combination therapies based on individual genomic alterations may lead to improved outcomes (Figure 1). High matching rates were found, primarily due to comprehensive molecular profiling, timely Molecular Tumour Board discussions, and rapid access to drugs (Sicklick et al., 2019; Dahabiyeh et al., 2022; Semreen et al., 2022; Dahabiyeh et al., 2023). Screenings that preceded cancer therapies to tailor therapy are in clinical trials, for instance, endometrial cancer falls that have distinct molecular profiles are actively being investigated with antagonists or inhibitors that are pathological molecular markers. Markers in this particular study play very similar roles in activating cell cycle progression to CDC25 as PTEN, PI3K pathway activate Cyclin D1 (Arend et al., 2018).

The potential for CDC25 as a therapeutic target arising from a precise analysis of various tumours can be related to cell cycle markers found in previous studies and their results. Molecular markers that are putatively ubiquitous with some cancers may not be directly related to the cell cycle, as in they do not directly act on the Cyclins, Cdks or checkpoint proteins involved, but they may affect the proliferation of cells or indicate an irregularity in cell cycle regulation as the case is for PD-L1 in various cancers (Schulz et al., 2021) even those with more complex ontology (Banchereau et al., 2021). Precision medicine studies involving machine learning algorithms were able to decipher from complex and heterogenous genomic (Tang et al., 2023) data the potential efficacy of using the PD-L1 inhibitors on various cancers based on molecular determinants of the tumours via transcriptional profiling using RNA sequencing under immune checkpoint inhibitors (Banchereau et al., 2021; Chen et al., 2021). Results from this can be used to inform and direct treatment-responsive patients to those respective therapeutic agents (Cristescu et al., 2018) or radiotherapeutic procedures (He et al., 2020; Johannet et al., 2021). The checkpoint PD-1 and PD-L1, similar to CDC25 have been observed to sustain long term remission by utilizing personalized and genomic based targeting specifically in melanoma (Axelrod et al., 2018; Petrova et al., 2020).

An advantage of some of the aforementioned conjugation of *in silico* and *in vitro* methods mentioned above is that new patterns and markers can be retrieved from previously archived data that may be obscured by the noise of redundant pathways (Salameh et al., 2022). The image analysis of the microarrays, histographs and tumour sections are closer to being linked than previously thought using these machine learning methods to link the pathophysiological characteristics of those objects to the molecular profiles (Chua et al., 2021). Trials that used biomolecular markers such as epidermal growth factor receptors targeted after genomic analysis of patients' tumor profiles', observing a significant result without chemotherapy using immunotherapy (Hainsworth et al., 2018). In corollary, these same methods can be used to analyze drug libraries to ascertain whether chemical structures and properties of archival drugs can be repurposed for novel chemotherapeutic purposes (Issa et al., 2021; Tao et al., 2021) and with the expansion of click chemical construction many more can be constructed using the existing functional backbones to overcome resistance (Tao et al., 2021; Vartak et al., 2023; Chowdhary et al., 2024), which leaves hope that there may already be an anti-CDC25 agent or a precursor that is awaiting further testing or reconfiguration.

5 Cell cycle regulation and CDC25 phosphatases

5.1 Overview of the cell cycle and its importance in cellular function

The cell cycle in somatic cells is divided into two main parts, interphase in which the cell replicates DNA, growth factors and proteins required for the subsequent mitotic division to create the

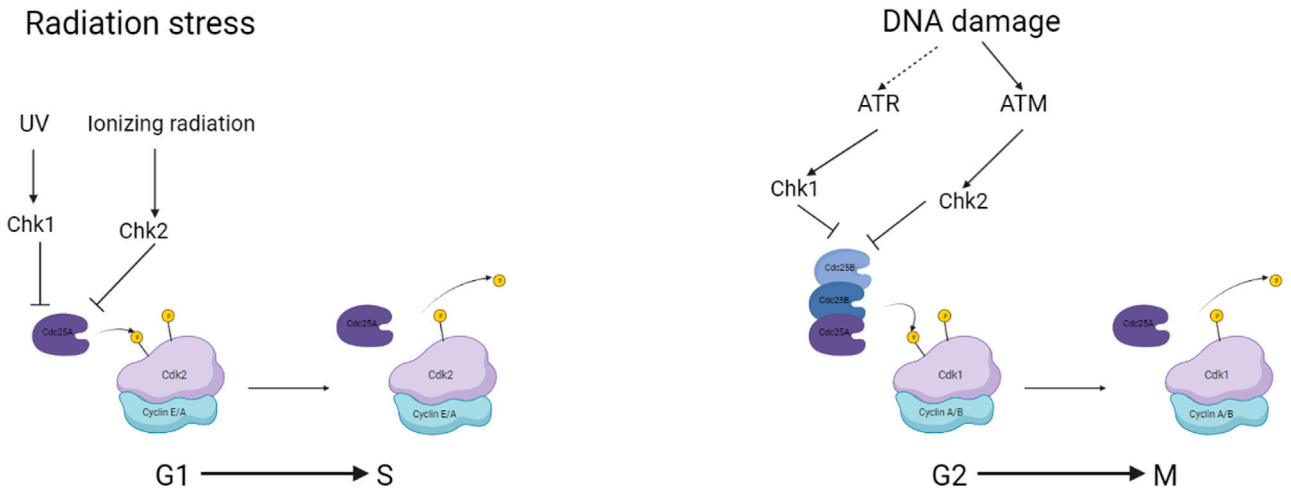

FIGURE 2
a simplified overview of checkpoints associated with CDC25 and the regulation during genomic damage. Created with BioRender.com.

two daughter cells. Some cells enter the cell cycle from a G0 or gap phase after interacting with a mitogenic factor. The cells will then enter G1 followed by a phase specifically for replicating DNA, an S phase a G2 phase where checks on the integrity of the replicated DNA occur and finally the cell's mitotic M phase and cytokinetic division. Between each phase, there are figurative checkpoints that are regulated by the activation and complexing of CDKs with Cyclins (Figure 2). The regulation and control of cells determined to enter the cell cycle begin at the G1 phase with the interaction of Cyclin D with CDK4/6. This CDK-Cyclin complex interacts with Retinoblastoma protein (RB) releasing chromatin remodelling enzymes and transcription factors triggering the expression of downstream CDK. The activation of these signals and regulating proteins such as RB is achieved through the phosphorylation of amino acid residues. CDK-Cyclin complexes are both activated and inactivated by similar mechanisms. The activity of the complexes is repressed mainly by the phosphorylation of Threonine14 and Tyrosine15 catalyzed by WEE1 and MYT1 kinases (Agius et al., 2015). The activation required for cell cycle progression is completed by the protein phosphatase CDC25.

5.2 Explanation of CDC25 phosphatases and their role in cell cycle progression

As with many of the mitotic factors and cell cycle regulation control proteins, CDC25 was first discovered in the fission yeast *Schizosaccharomyces pombe* (Hoffmann and Karsenti, 1994) as a positive regulator of CDC2 now known as CDK1 (Russell and Nurse, 1986). CDK1 activation ensures that cells within interphase can commit to mitotic division (Bretones et al., 2015). As mentioned before the role of CDC25 as a protein phosphatase is to remove the inhibitory phosphate group from Tyr15 of CDKs involved in progression through the cell cycle checkpoints. Activation of CDK through binding with Cyclins and potential phosphorylation by protein tyrosine kinases (PTK) leads to activation and progression through the cell cycle leading to mitotic entry and continuation of the cell cycle. The constitutive activation of CDK-Cyclin complexes has been implicated in the initiation of many cancers, with constitutively active CDK-Cyclin complexes ensuring continuous kinase activity and activation of cell cycle factors such as downstream CDKs and chromatin remodelling enzymes. Leading to an aberrant cell cycle and unregulated cell division, forming a neoplasm that may progress into a cancerous cell mass if left unchecked.

CDC25 not only has an effect on the activation of the CDK-Cyclin complex but another regulator of cell cycles, the Raf/MEK/ERK pathway. In prostate cancers, CDC25A inhibitors were used and found to inhibit the downstream activation of MEK with CDC25A directly (Nemoto et al., 2004). The Raf/MEK/ERK pathway is activated by growth factors interacting with growth factor receptors resulting in the downstream activators of transcription factors such as AP-1, an important trans-acting factor involved in regulating the expression of cell proliferation, differentiation, and apoptosis. CDC25A was hypothesized to remove the inhibitory phosphate group from Raf as seen with the hyperphosphorylation of Raf after the minimal application of CDC25 inhibitor, NSC 95397 or NSC 672121 (Nemoto et al., 2004). Specifically, a putative proto-oncogene that has been more directly implicated in cancer and connected to the Raf/MEK/ERK pathway is the transcription factor c-Myc, inducing DNA replication by binding to activator sites (Bretones et al., 2015).

The ability of CDC25 inhibitors to cause cell cycle arrest and death in cancer cells makes them promise as cancer treatment agents. This is accomplished by inhibiting CDK activation, which inhibits cell division and regulates the cell cycle. Because normal cells are less dependent on CDC25 for cell division and cell cycle control than cancer cells, the mechanism of action of CDC25 inhibitors renders them less harmful to them.

5.3 Dysregulation of CDC25 phosphatases in cancer and its impact on tumour formation

Dysregulation and constitutive expression of CDC25 have shown to be a constitutional mechanism in some cancers, with an overexpression observed and implicated in clinical outcomes of breast, ovarian and colorectal cancer patients (Figure 2). In the case of ovarian cancer studies, found that the overexpression investigated using immunohistochemistry that the poor prognosis had a link to the overexpression of CDC25A and CDC25B in a sample of 106 patients (Broggini et al., 2000). Additionally, breast cancer resistant to ionizing radiation was found to overexpress CDC25A (Löffler et al., 2003). CDC25A and CDC25B showed strong correlations to high-grade lymphoma reported as aggressive (Kristjánsdóttir and Rudolph, 2004). Cdc25A and Cdc25B were also overexpressed in non-Hodgkin's lymphoma and various other cancers, including oesophageal, gastric, lung, thyroid, and head and neck cancers (Kristjánsdóttir and Rudolph, 2004). Exclusive overexpression of CDC25A in hepatocellular carcinoma is rare (Xu et al., 2003), while pancreatic ductal carcinoma and gastric carcinomas exclusively overexpress Cdc25B (Kristjánsdóttir and Rudolph, 2004).

All three isoforms of CDC25 would be considered proto-oncogenes, with the overexpression or constitutive activation leading to premature entries into either the S or M phase of the cell cycle. The role of Myc protein in overexpressing CDC25 is rather complicated and according to the literature quite contested. It appears that the role of Myc in overexpressing CDC25 follows cancer-specific patterns. In lymphoma and certain lung cancers, there was a clear correlation between the overexpression of Myc and CDC25 (Hernández et al., 1998). Some speculate that the interaction and overexpression of CDC25 in relation to MYC is context-dependent, as both CDC25A and CDC25B proteins have Myc target sites (Galaktionov et al., 1996; Kristjánsdóttir and Rudolph, 2004).

A big discovery was the fact that CDC25 overexpression did not drive cell proliferation with an absence of correlation found between the expression and rate of proliferation in many studies (Hernández et al., 1998; Cangi et al., 2000; Miyata et al., 2000). This is most likely due to the involvement of other necessary growth factors and signalling cascades required for the expression of cell cycle genes. It does appear that CDC25 overexpression allows for bypassing checkpoints involved in checking for genomic damage before entering S and M phases (Kristjánsdóttir and Rudolph, 2004).

6 Precision medicine and molecular profiling

6.1 Definition and purpose of precision medicine in cancer treatment

Precision medicine is a cutting-edge medical strategy that examines the genetic, genomic, epigenetic, and proteomic changes present in cancer cells using cutting-edge molecular profiling technology. Precision medicine enables oncologists to choose tailored therapies that directly interfere with the aberrant signalling pathways responsible for tumor genesis and progression by discovering particular biological drivers of cancer growth and survival (Naithani et al., 2021). Precision medicine therapies are intended to specifically attack cancer cells while preserving healthy organs, hence lowering treatment-related adverse effects, in contrast to standard chemotherapy, which broadly targets rapidly dividing cells.

Personalized methods are being made critical to cancer research due to improvements in diagnostic accuracy, optimizing treatment choices, and generally improving patient outcomes. Precision medicine can uncover specific genetic variations or modifications fuelling the growth of cancer cells by examining a patient's tumour at the molecular level. This knowledge can help lead to the development of tailored medicines such as individual immunotherapy that directly target personalized and distinct immunophenotypes (Zeggini et al., 2019) that can be formulated from the previously mentioned molecular aberrations, boosting the likelihood of a positive response (Naithani et al., 2021). Furthermore, precision medicine promotes the development of biomarker-driven clinical trials, which aim to test the efficacy of new medicines on patient subgroups with similar genetic or molecular traits (Kong et al., 2022). This approach enables researchers to find novel therapeutic targets and hasten the development of innovative medications that are more effective and less hazardous.

With the explosion of machine learning models and AI-driven methods for pattern recognition in large biological data sets it appears that precision medicine may only become more granular and effective by detecting the relevant and appropriate markers associated with detection and for relating patients to the correlating effective treatment (Bhinder et al., 2021; Nayarisseri et al., 2021). In addition to molecular identification of personal biomarkers, archival images retrieved from radiotherapy can be used to glean the efficacy of other radiological treatments for personal cancers (Viswanathan et al., 2014). By incorporating radiomics into cancer research, we may combine imaging data with other genetic profiling methods to better understand the heterogeneity of the tumour and develop individualized therapy plans encompassing post-procedure lifestyle and care (Wang et al., 2022). As it provides a non-invasive and therapeutically accessible way to evaluate the genetic properties of tumours and track treatment outcomes over time, this technology is crucial to the field of precision medicine.

6.2 Introduction to molecular profiling techniques for identifying molecular alterations in cancer cells

Cancer is a challenging and heterogeneous pathology that is triggered by genetic and molecular changes that impair regular cellular functions and cause unchecked cell growth and proliferation. For the purpose of improving cancer research, diagnostic, and treatment methods, it is crucial to comprehend the molecular environment of cancer cells. The development of comprehensive molecular profiling tools has advanced significantly over the past two decades, revolutionizing our ability to identify the genomic, transcriptomic, epigenomic, and proteomic changes found in cancer cells (Figure 3). Using previous data points precision medicine has also been able to identify radiomic response of certain

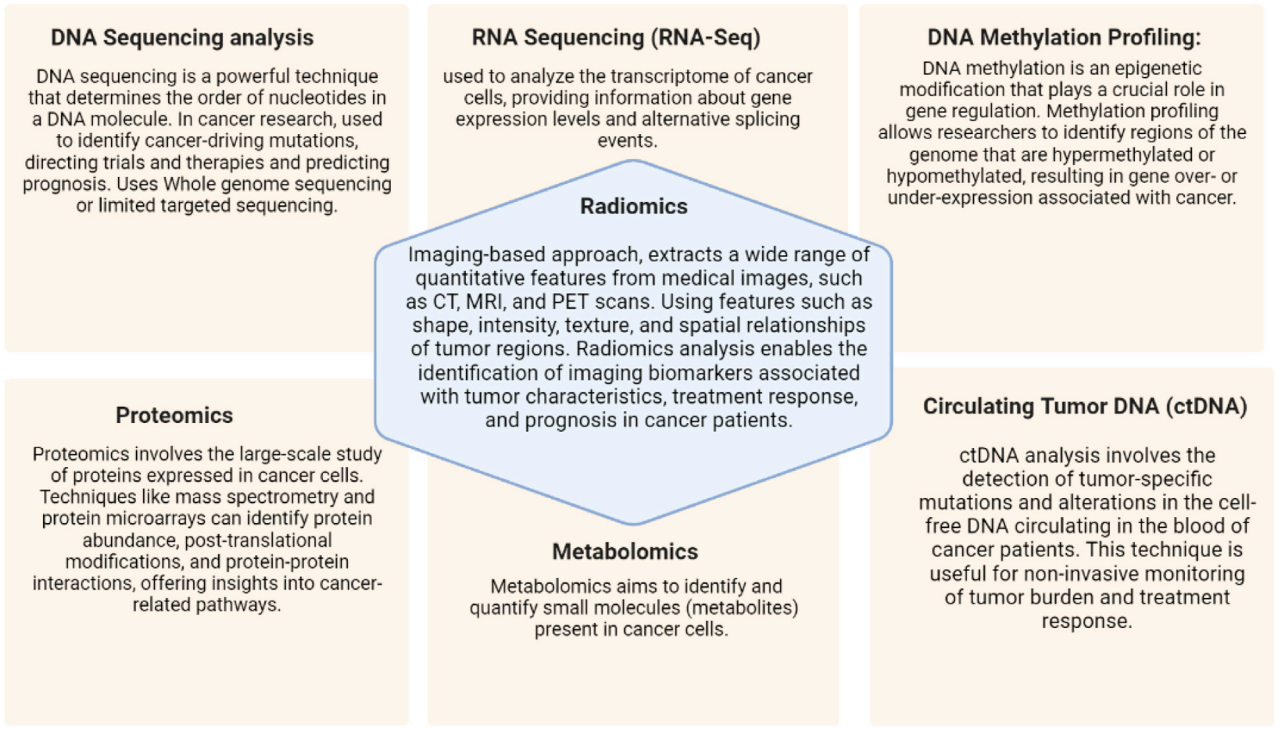

FIGURE 3
Molecular profiling techniques and imagining in precision oncology.

molecular mutations inherent in some populations of cancer, easing both the load on patient and healthcare providers (Rosario et al., 2018).

Liquid biopsies, such as circulating tumor DNA and circulating tumor cells, present promising results for non-invasive tools for detecting and monitoring Small cell lung carcinoma, providing insights into tumor heterogeneity and potential therapeutic targets (Meijer et al., 2022). Non-invasive methods in combination with personalized medical techniques, increasing turnaround times and detection for particularly heterogeneous cancer types such as colorectal cancer and prostate cancer (Vandekerkhove et al., 2019; Malla et al., 2022). Along with genomic and deep learning analyses biomolecular markers, potentially CDC25, can be used as predictive measures for predictive biomarkers for treatment outcomes and recurrence risk in cancers, emphasizing the need for further multicentred investigations (Nakano et al., 2023). Heterogeneity in major cancers such as breast and gastric cancers poses a major hurdle for therapy, with precision medicine it is proposed that it will be significantly easier to choose patients for certain treatments (Garrido-Castro et al., 2019; Wang et al., 2019). CDC25 can be identified from single-cell analysis due to cytoplasmic localization of the phosphatase in rapidly proliferating and continual cells (Gabrielli et al., 1996).

Other Omics technologies other than radiomics have utilities for either the identification of targets for developing and formulating small molecule inhibitors. Analysing proteomic alterations in protein expression or post-translational modifications throughout checkpoint inhibitor therapy facilitates the assessment of therapeutic efficacy and prognostication of patient outcomes. Proteomic scrutiny alongside learning platforms enables thorough and dynamic monitoring of shifts in protein profiles across different graded cancers (Mehlich and Marusiak, 2022; Monsivais et al., 2023), providing critical insights into treatment responsiveness and the emergence of resistance mechanisms (Yu et al., 2023). With the repository of microarray data, comparative and exploratory algorithms checking for overexpression between cancerous and benign tissue can illuminate subjects for treatment, such as checkpoint inhibitors. A similar basis can be used for CDC25 as was used for MCM6 (mini-chromosome maintenance), a vital protein for chromosome stability. Proteomics has linked other members within this protein family, finding overexpression between cancerous and normal tissue, particularly in bowel cancer (Wang et al., 2023). The utility of proteomics was not just singled out to this check-point inhibitor, Proteolysis targeting chimera D6 in triple-negative breast cancer along a CDC25-CDK1 axis was found by comparing triple-negative breast cancer cell line response against D6 (Wu Y. et al., 2024).

Later on in this review, we will expand on the subject of quinones, a derivative of which 6-isomer of 5,8-quinolinedione performed cytotoxically well in colorectal cell lines. A combination of proteomic and phosphorylation profiling was able to demonstrate the efficacy (Narwanti et al., 2023), presenting other avenues for trialling small molecule inhibitors. Within the precision medicine parameter integration of proteomics, particularly concerning small molecule inhibitors, empowers clinicians and researchers with intricate molecular insights. Through mass spectrometry-based techniques, protein expression

levels, post-translational modifications, and interactions are comprehensively analyzed. Using machine learning models and evolving functional and spatial mathematical modelling reveals cellular pathways that are vital for stemminess a crucial property of understanding cancer therapeutics (Plattner et al., 2023). This synthesis aids in the tailored selection, validation, and optimization of therapeutic strategies, advancing the realization of precision medicine's promise for personalized and effective patient care (He and Wang, 2023; Plattner et al., 2023; Srivastava et al., 2024).

Other revolutionary technological advance representing a synergy of large cancer data analysis and diagnosis/discovery is metabolomics, using low molecular weight metabolites produced by various aberrant cellular processes (Carapito et al., 2024). Underpinned by the reality that cancer cells have different metabolic profiles than normal cells and can be used to find biomarker signature. Glutamine, a pivotal metabolite, acts as a versatile substrate intricately involved in a myriad of biosynthetic pathways critical for the unchecked proliferation of cancer cells. Serving as a fundamental building block fuelling diverse cellular processes, encompassing the synthesis of nucleotides, amino acids, and fatty acids essential for sustaining the rapid growth and expansion characteristic of malignant cells making the catabolic enzyme glutaminase a valuable target for cancer therapeutics (Cyriac and Lee, 2024). Other profiles that are used for research purposes and hopefully another facet for therapeutic discovery is the lipid profile (Wu Q. et al., 2024) and kynurenine resulting from liver metabolism, demonstrating results with a high precision and specificity using machine learning models (Wu Q. et al., 2024) illuminating other potential targets for cancer treatment or diagnosis by finding enzymes and differentially regulated genes within their respective pathways (Dai et al., 2023). Another subset of metabolomics, volatile organic compound–omics presents another potentially valuable avenue of research using the volatile profile of human exudates or excretions in, for example, bladder cancer marker identification (Carapito et al., 2024).

The traditional molecular techniques used in conjunction with the recent machine learning approaches have broadened the knowledge gap in some of the more difficult and more ontologically convoluted cancers such as prostate cancer. Most notably deep neural networks, a class of machine learning algorithms designed to model complex patterns and relationships in data by using multiple layers of interconnected artificial neurons, can predict Gleason scores from visuals of prostate cancers taken during histopathology, deep neural networks have so far produced excellent results (Nagpal et al., 2020).

7 Genetic variations in CDC25 phosphatases

As mentioned above there are three major isoforms of CDC25: CDC25A, CDC25B and CDC25C. The molecular weights of the three CDC25 isoforms range from 53 to 65 kDa. CDC25A and CDC25B comprise 524 and 580 amino acids respectively whilst CDC25C consists of only 473 (Brenner et al., 2014). The CDC25 protein structure is separated into two major regions: the N-terminal area and the C-terminal region. The N-terminal region is quite varied, including phosphorylation and ubiquitination sites governing phosphatase activity. The catalytic site is located at the C-terminus, which as mentioned before is quite consistent between the isoforms (Sur and Agrawal, 2016). The CDC25 family's highly conserved area with uncertain function is the adjustable cysteine residue, Cys484, which is situated in a cleft binding to a sulphate group (Reynolds et al., 1999). Oxidation of active site cysteine has been suggested to be a part of a checkpoint for increasing the oxidation state within the cell, ROS attacking the cysteine leads to a triggering of this checkpoint (Kristjánsdóttir and Rudolph, 2004). Hotspots, considered essential for substrate identification, are situated around 20–30Å away from the active site (Sohn et al., 2004). All CDC25 isomers contain conserved catalytic domains but very different regulatory regions. Regulatory areas are subjected to alternative splicing events, which result in two variants for CDC25A and five each for CDC25B and CDC25C (Baldin et al., 1997; Wegener et al., 2000). The phosphatases' intracellular location and turnover are determined by the non-catalytic domain. The general structure outlined has strong similarities to other dual specificity phosphatases involved in cellular communication and cell cycle regulation, which will have implications for inhibitor generation.

CDC25A is activated during the G1 phase of the cell cycle and is responsible for driving the cell through the G1 checkpoint and into the S phase. It is degraded by proteolysis via the ubiquitination mechanism at the end of mitosis (Donzelli et al., 2002). CDC25B is expressed during the G2 phase of the cell cycle and stimulates CDK1, which is necessary for mitosis to begin (Gabrielli et al., 1996). CDC25C is similarly involved in the mitotic entrance, but it is controlled by the checkpoint kinase CHK1 and is activated only after DNA damage has been repaired (Frazer and Young, 2012).

CDC25 activity is closely controlled by a variety of processes, including phosphorylation, proteolysis, and gene expression regulation. Checkpoint circuits that respond to DNA damage and other stimuli to limit cell cycle advancement until the damage is repaired also control CDC25 activity. Phosphorylation is one of the essential CDC25 regulatory mechanisms. A variety of kinases, including CHK1, CHK2, and ATM, phosphorylate CDC25 (Sur and Agrawal, 2016). CDC25 phosphorylation can either promote or inhibit its action, depending on the location phosphorylated and the kinase involved. For example, phosphorylation of CDC25 in response to DNA damage by CHK1 or CHK2 limits its function, preventing the cell from starting mitosis until the damage is repaired (Sur and Agrawal, 2016).

Proteolysis regulates CDC25 as well. After mitosis, the anaphase-promoting complex/cyclosome (APC/C) targets CDC25B for degradation, preventing it from activating CDK1 and sending the cell back into the G1 phase. Similarly, during the S phase, the SCFβ-TrCP complex targets CDC25A for degradation, preventing premature entrance into mitosis. Degradation is achieved by the phosphorylation of three serine residues by CHK1/CHK2 (Boutros et al., 2006). The MAPK5 pathway, JNK and p38 pathways, and checkpoint kinase Chk1 all target CDC25 phosphatases, particularly CDC25B, for degradation or inhibition, leading to cell cycle arrest or delay. CDC14A phosphatase can also inhibit the catalytic activity of CDC25B by dephosphorylating it, preventing premature entry into mitosis (Sur and Agrawal, 2016).

The final member of the CDC25 cohort, CDC25C, is inactivated by various protein kinases and phosphatases. Chk1, Cds1/Chk2,

TABLE 1 Mode of action and mechanism of potential anti-cancer compounds with the limitations/hurdles with the application for treatment. Progress in trials was either inferred from research papers or direct results from ClinicalTrials.gov.

Name of Cdc25 inhibitors	General inhibitor type	Molecule type	Mode of action	Sources
NSC-663284	Small molecule inhibitor	Quinolinedione	Blocking the binding of CDC25A	Lazo et al. (2002)
IRC-083864	Small molecule inhibitor	Bis quinone	Binding to CDC25B	Brezak et al. (2009), Sarkis et al. (2017)
NSC-95397	Small molecule inhibitor	Quinone-based	Binding to all isoforms	Peyregne et al. (2005)
BN82685	Small molecule inhibitor	Quinone-based	Direct binding to CDC25	Brezak et al. (2009)
SN-38	Indirect inhibitor	active metabolite of irinotecan	Activation of CHK1/2	Ditano et al. (2021)
2-fluoro-4-hydroxybenzonitrile	Small molecule inhibitor	nitrile derivative	CDC25B catalytic domain binding	Lund et al. (2015)
Genistein	natural product, an indirect inhibitor	isoflavone compound (legume derived)	Activation of CHK1/2	Brenner et al. (2014)
Bozitinib	small molecule inhibitor	Amino pyrimidines	Inhibits activation of CDC25	Lavecchia et al. (2010)
UPD-140	Small molecule inhibitor	Naphthoquinone	Inhibits CDC25A	Kabakci et al. (2019)
UPD-176	Small molecule inhibitor	Naphthoquinone	Inhibits CDC25A	Kabakci et al. (2019)
UPD-172	Small molecule inhibitor	Naphthoquinone	Inhibits CDC25A	Kabakci et al. (2019)
UPD-596	Small molecule inhibitor	Naphthoquinone	Inhibits CDC25A	Kabakci et al. (2019)
UPD-1419	Small molecule inhibitor	Quinone	Inhibits CDC25A	Kabakci et al. (2019)
UPD-1416	Small molecule inhibitor	Quinone	Inhibits CDC25A	Kabakci et al. (2019)
UPD-795	Small molecule inhibitor	Naphthoquinone	Inhibits CDC25A	Kabakci et al. (2019)
Menadione	Small molecule inhibitor	Quinone	Inhibits CDC25A, B, C	Abdelwahab et al. (2022)
Cpd-5, [2-(2-mercaptoethanol)-3-methyl-1,4-naphthoquinone]	Small molecule inhibitor	Quinone	Inhibits CDC25A, B, C	Abdelwahab et al. (2022)
Cpd-42	Small molecule inhibitor	Vitamin K derivative	Inhibits CDC25A	Abdelwahab et al. (2022)
Cpd-5, derivative 6	Small molecule inhibitor	Vitamin K derivative	Inhibits CDC25A, B, C	Abdelwahab et al. (2022)
Cpd-5, isomer 7	Small molecule inhibitor	Vitamin K derivative	Inhibits CDC25A, B, C	Abdelwahab et al. (2022)
NS1' Protein	natural product, an indirect inhibitor	Japanese Encephalitis Virus	Inhibits CDC25C	Li et al. (2021)
SV37	Small molecule inhibitor	coumarin-quinone derivative	Inhibits CDC25B, C	Abdelwahab et al. (2022)
Caulibugulones A-F	natural product, direct inhibitor	marine bryozoan *Caulibugula intermis*	Inhibits CDC25B	Abdelwahab et al. (2022)
Albendazole	Small molecule inhibitor	Benzimidazole	Inhibits CDC25A	Di Fusco et al. (2020)
Shikonen	Natural product, direct inhibition	Naphthoquinone, *Lithospermum erythrorhizon*	Inhibits CDC25C	Zhang et al. (2019)
APE	Natural product, Indirect inhibition	Annurca apple, polyphenol extract	Upregulate phosphor-CDC25C	Martino et al. (2019)

c-TAK1 kinase, and JNK are involved in the phosphorylation of CDC25C at Ser287, Ser168, and Thr138 (Liu et al., 2020), leading to the inactivation of CDC25C (Boutros et al., 2006; Sur and Agrawal, 2016). Phosphatases like PP1, PP2A, and hCDC14B dephosphorylate CDC25C to activate it, while the PP2A-B56δ complex negatively regulates CDC25C activity by dephosphorylating Thr138, leading to its exit from mitosis (Sur and Agrawal, 2016).

CDC25 expression is also controlled at the transcriptional level. The E2F family of transcription factors, which are activated by the RB protein, can promote cell cycle advancement by inducing the expression of CDC25A and CDC25B (Vigo et al., 1999; Sur and Agrawal, 2016). In contrast, the tumour suppressor protein p53 can limit CDC25C expression, preventing cells from entering mitosis in response to DNA damage.

8 CDC25 inhibitors in precision cancer medicine

8.1 Overview of CDC25 inhibitors and their classification

Many CDC25 inhibitors are currently being investigated for clinical use against tumorigenesis or the metastasis of more developed cancers. The initial testing as with all treatments starts with an *in-vitro* trial on the relevant cancer cell lines or tissue, and if proven to have successful inhibitory effects on cancer would then follow onto *in vivo* using graft models of humanized mice and then the clinical trials (Liu et al., 2020). However, this poses a challenge with CDC25 as there are 3 forms of the protein with slightly different structures and it has been shown that suppression of one may suppress the tumour growth but may not be sufficiently powerful to move forward into clinical testing and development of a ready-for-market treatment. The isoform predicament can also be seen as a blessing in disguise as some research shows that all three interactions are required for entry into M phase (Sur and Agrawal, 2016). In this section of the review, the origins, effects, and results of several putative cdc25 inhibitors will be summarized (Table 1). The three general types of CDC25 cancer inhibitors include small molecule inhibitors, peptide-based inhibitors, and natural product inhibitors.

The discovery of dysidiolides in 1994 marked the beginning of the discovery of natural and synthetic compounds that modulate CDC25 family proteins. Many compounds have been described in recent reviews, and more patents and studies have reported new interacting molecules for CDC25 phosphatase inhibitors. Much effort has been focused on CDC25 phosphatase inhibitors in the past 5 years (Lavecchia et al., 2010). Dysidiolides are part of the small molecule inhibitor group, inhibiting CDC25 by binding to their catalytic site. These small molecule inhibitors are usually found using pharmacokinetic modelling studies. Knowledge of the crystalline and genetic structure has been evaluated and confirmed, opening up avenues for these small molecule inhibitors to be found using complex modelling and kinetic energy studies using a specific isoform of CDC25 and a repository of chemicals from the National Cancer Institute (Lazo et al., 2002). These inhibitors become pillars from which derivatives are constructed, using them as a backbone which has shown functional effectiveness for exploration against a CDC25 structural query, as in the dysidiolide case (Koch et al., 2004; Shimazawa et al., 2004).

Multiple cellular mechanisms can be invoked when dealing with CDC25 inhibition. The action may be direct by binding to CDC25 hotspots (Lavecchia et al., 2010), or indirectly through upstream inhibition, extant phosphorylation of CDC25 species (Lu et al., 2012) halting cell cycle progression, and inducing apoptosis (Martino et al., 2019). In terms of upstream effectors, regulating kinases such as JNK in cancers that have been observed to have perturbed activity in various cancer tissue, especially the invasive triple negative MDA-MB-231 breast cancer cells, are targets for many exploratory research groups for shutting down cell cycle progression (Sur and Agrawal, 2016; Martino et al., 2019). Another critical anti-oncogenic cellular pathway that should be considered within the discovery of cell cycle inhibitors are those that additionally trigger apoptosis. Polyphenols that are found in apple skins demonstrated, namely, APE was found to not only arrest cell cycle in G2 phase in CDC25C dependent manner but triggered ROS dependent intrinsic and extrinsic apoptosis in MDA-MB-231 (Martino et al., 2019). Once verified by *in-vitro/in-vivo* assays CDC25 inhibitor backbones that are not specific should be investigated, rather than discarded as there are programs using single cell transcriptomics in development that integrate patient-derived data to inform on drug combinations, such as ComboSC and the work of Berlow et al. (Berlow et al., 2019; Tang et al., 2023)

Modelling for active site binding mediated inhibition is difficult in CDC25 due to its unique structure. CDC25 has a shallow active site region and the reactivity of the catalytic cysteine residue compound this issue of active site binding. As an alternative, attention has turned to identifying hotspots in the enzyme that are critical for interactions at the phosphatase-substrate interface. Thirteen residues in CDC25B were identified, and mutations in R488 and Y497 reduced both *in vitro* and *in vivo* dephosphorylation of CdK2-pTpY/CycA by Cdc25B. A deep pocket adjacent to the hotspots on CDC25B harbours amino acids essential for substrate-phosphatase interactions, making compounds that selectively bind in this pocket potentially effective in disrupting CDC25B enzyme activity (Lavecchia et al., 2010). General protein phosphatase inhibitors can be found to potentially sensitize tumors to immunotherapy and chemotherapy through targeting of larger conserved domains (Stanford and Bottini, 2023). Active sites of enzymes are not the only potential targets that can be modelled for, as has been shown in an analogous case of binding to dimerization or ligand binding sites (Vartak et al., 2023). When taking these sites into account it seems that the possibilities to affect cellular mechanisms are vastly more plentiful and potentially more fruitful than the tunnel vision of active site binding. Briefly, this allosteric targeting can be used in downstream targets of CDC25, such as cyclin E/CDK2 or CDK1 at Y15, thus preventing phosphatase and subsequent proliferation (Pellerano et al., 2017), although this may lead to adverse effects compared to targeting of the functionally narrow CDC25 (Chu et al., 2021).

8.2 Small molecule inhibitors

One of the earliest small molecule inhibitors that arose from these early searches was NSC-95397 which was reported to be a dual-phosphatase inhibitor (Lazo et al., 2002). NSC-95397, a para-naphthoquinone, was found to bind to all of the isoforms of CDC25 with a low IC_{50} for CDC25 in colon cancer cells of 9.9–18.6 μM. However, within this study, it was found that CDC25A expression was not downregulated compared to controls but was actually phosphorylating downstream ERK1/2 (Dubey et al., 2018). In the context of acute myeloid leukemia, CDC25 inhibitor NSC-95397 was shown to exert anti-proliferative effects on cell suspensions. Most likely due to the aforementioned cyclin/CDK inhibition in a cytogenic-state-dependent manner (Brenner et al., 2017). Suggesting that some of the established CDC25 inhibitors still require deeper validation to confirm and understand the mechanisms at play.

Similarly, NSC-663284 was found to inhibit in a similar magnitude to the traditional gemcitabine treatment in mouse model experiments, with an inhibitory dose of 5 mg/kg. The

efficacy of this CDC25 inhibitor for use in treatment was suggested to be influenced by several factors inherent to the molecule and the reaction with the metabolic reactions within the cells (Guo et al., 2007) and toxicity to the surrounding tissue. For instance, the quinone class of organic compounds, of which NSC-663284 is a part, has many members that effectively target and inhibit the enzyme activity of cdc25, binding with Tyr428, Arg482, Thr547 and Ser549 according to chemical binding simulations, (Guo et al., 2007; Ge et al., 2017), along with the additive effect of generating ROS that damage DNA, damages cellular superstructures and halts the progression of the cell cycle (Njus et al., 2023). Within the context of NSC-663284 it has been hypothesized that the inhibitor undergoes editing in order to form the electrostatic bonds to exert its inhibitory effects (Ge et al., 2017), if this modelling proves to be true with experimental data, the metabolite/edited product can be used in patient specific metabolic profiling of single cell colonies to evaluate the efficacy of NSC-663284 (Martino et al., 2019; Wekking et al., 2023).

CDC25 inhibitors could work indirectly on the deactivation of CDC25 by using endogenous regulation pathways and overexpressing/activating them leading to downregulation of CDC25. IRC-083864 is a strong CDC25 family protein inhibitor with low nanomolar activity and no inhibitory action on other phosphatases. Preventing mitosis and boosting CDK1 phosphorylation significantly suppressed tumour cell growth and changed cell cycle progression. IRC-083864 also caused apoptosis in tumour cells produced as spheroids and inhibited the development of human MIA PaCa-2 and LNCaP xenografts in animal models. While greater doses resulted in animal body weight loss, no harm was seen at lower doses (Brenner et al., 2014). The strong potency and anti-tumour activity of IRC-083864 promotes its further development as a viable treatment for drug-resistant malignancies. Another mechanism that can be used is ROS-mediated damage which would lead to the deactivation of CDC25 by phosphorylation via CHK1/2 (Kristjánsdóttir and Rudolph, 2004; Sur and Agrawal, 2016). NSC 119915, an irreversible inhibitor of this vein of mechanics, creates intracellular ROS in cells and stops them in the G0/G1 stage and G2/M stage of the cell cycle by inhibiting the two CDC25A and CDC25B. In previous studies, Genistein as an inhibitor has demonstrated a restriction of K562 leukemia, PC-3 prostate cancer, and MCF-7 breast cancer cell line progression considerably (Brenner et al., 2014).

Many novel small molecule inhibitors have been synthesized working off of *in silico* modelling of CDC25 inhibitors, considering structural, steric and isoformic activity and interactions. An issue can arise where there are too many small molecule inhibitors that are formulated from these methods and arise from too narrow a scope, but this is probably a good problem to have in relation to relying on possible ineffective and "brute-force" generalized therapies. The issue can be circumvented by using high-throughput methods but will experience a bottleneck at the animal testing stage, an invaluable stage that cannot be replaced (Tanoli et al., 2021). Suggesting that inhibitors to CDC25 activity may not need to specifically target the CDC25 protein but can affect the physiological effects associated with CDC25 involving pathways including mitotic spindle assembly (Cazales et al., 2007). For instance, the novel inhibitor WG-391D tested in ovarian cancer mouse models, presented advantageous effects to inhibiting tumor growth by down-regulating CDC25B (Xiao et al., 2019).

8.3 Natural product

The utilization of natural products and plant extracts has become more significant in the field of cancer research, primarily due to their potential as supplementary treatments. These substances present unique opportunities for the development of innovative therapeutic approaches. The extensive array of bioactive chemicals found within this particular subject matter offers promising prospects for precise and focused interventions. The potential for enhanced cancer management lies in the utilization of the synergistic effects between natural medicines and conventional therapy (Al-Eisawi et al., 2022a; Al-Eisawi et al., 2022b; Eldesouki et al., 2022; Bou Malhab et al., 2023; Tarawneh et al., 2023).

Another indirect and small molecule inhibitor of CDC25, from natural components, is Genistein. Genistein, a tyrosine kinase inhibitor, activated p38 in human mammary epithelial cells, involved in the downregulation of CDC25C levels and phosphorylation of CDC2 leading to an arrest of the cell cycle at the G2/M checkpoint (Frey and Singletary, 2003). Activation of p38, a mitogen-activated protein kinase, is essential for genistein-mediated growth inhibition, although it is not the only requirement. Additionally, genistein induces a G2 arrest by impairing the Tyr15 dephosphorylation of CDC2 via CDC25C, likely through a genistein-induced activation of CHK2 (Ouyang et al., 2009). Moreover, downregulation of the CDC25 level through p38 participation may be an important way to impair its actions and a meaningful act in G2/M checkpoint regulation. The investigation of the activities underlying the genistein inhibition or other agents on proliferation will need further clarification, considering the responses of other mitogen-activated protein kinase pathways, especially when considering different cytological sources (Frey and Singletary, 2003).

Even though there are beneficial result for single target CDC25 natural product inhibitors there is a greater need in our opinion for a multifaceted inhibitory attack on multiple isoforms of CDC25, that does not lose any specificity. In this regard natural products, such as Shikonin, could be of greater utility as observed in other phosphatase inhibitor combinations accompanying treatment granted that there is synergy and not agonistic effects (Marciniak et al., 2023). The research shows that the target effect of cell cycle checkpoint inhibitors may be expanded to inducing apoptosis. In the case of metabolite polyphenols APE retrieved from apple skins it was found to induce both apoptotic pathways, observed in the depletion of procaspase 3, 8 and 9, as well as arresting the cell cycle in a mimic to CDC25C action inhibition. However the main mode of action of the increase in inactivate phosphor-CDC25C was through ROS mediated cellular pathways indirectly acting on cell cycle progression rather than directly on CDC25 as is seen with most of the small molecule inhibitors (Martino et al., 2019). Offering additional anti-neoplastic effects that can be implemented in patient data driven combinatorial therapies, mainly through single cell metabolic profiling or a multiplexed omics platform which have

been implemented with combinatorial therapies for ROS inducing agents (Huttunen et al., 2023) or immune checkpoint inhibitors (Massa et al., 2022). Given the results in other types of cellular pathway enducers and inhibitors there is hope that these results can also be translated to cell cycle checkpoint inhibitors such as CDC25 for sub-groups of patients identified as susceptible to the treatment.

8.4 Antisense oligonucleotides

In contrast, antisense oligonucleotides target the CDC25 mRNA and block its expression and synthesis much like the siRNA or miRNA mechanism of downregulation of translation. This is beyond the scope of this review, but the research shows that antisense oligonucleotides of CDC25 are effective in inhibiting cell cycle continuation through G1 to S. However, within the review search, it appears that researchers are studying affecting or upstream proteins to CDC25 which may suggest that post-transcriptional silencing of CDC25 directly is not a viable path for therapeutics due to the penetration of the silencing to the cell cycle. In theory, this could work but would require accurate and reliable delivery systems to specifically target neoplastic tissue. Allowing silencing through these means opens up combinatorial avenues of treatment with existing methods (Gharaibeh et al., 2021). One of the many advantages of using relatively novel strategies when it comes to tackling cancer is the hope that it may reduce multiple-drug *resistance* that can otherwise be observed with the more traditional and long-used chemotherapy cocktail of drugs. In *Xenopus laevis* studies of CDC25A antisense oligonucleotides, eggs injected with the oligonucleotide were found to prolong the presence of the repressed phosphorylated Cdk1 in a dose-dependent manner up to a threshold concentration (Yoshitome et al., 2019), suggestive of a potential therapeutic dose for inhibiting aberrant CDC25A activity. Further explored in hepatocellular carcinoma where the overexpression was related to poor prognosis, CDC25A antisense oligo demonstrated the ability to decrease invasiveness but more importantly reduced cell proliferation and progression (Xu et al., 2008). Although from the research articles found this field is not being explored as much recently relative to the aforementioned inhibitor types.

9 Challenges and future directions

9.1 Addressing challenges in developing specific and effective CDC25 inhibitors

However, the development of CDC25 inhibitors as a cancer treatment has been challenged by several limitations and obstacles. One of the challenges in the development of CDC25 inhibitors is the specificity of the inhibitors for different copies of CDC25 and their functions in maintaining the integrity of DNA on top of the cell cycle progression. CDC25A, CDC25B, and CDC25C have slightly different roles in the cell cycle; CD25A is involved in the G1/S transition activating the CDK2 promoting DNA replication entry in S-phase, CDC25B Therefore, it is important to develop inhibitors that are specific for each isoform of CDC25 to minimize off-target effects. A related challenge arises from the potential toxicity of CDC25 inhibitors due to the role CDC25 has in the regulation of the cell cycle, inhibiting its activity and leading to cell death. However, this can also affect normal cells and tissues, leading to side effects such as bone marrow suppression, gastrointestinal toxicity, and neurotoxicity. Therefore, it is important to develop CDC25 inhibitors that are selective for cancer cells and have minimal toxicity to normal cells.

Research has shown that the inherent structure and mechanism of action of CDC25 could also play a role in limiting the efficacy of CDC25 inhibitors. For one their broad substrate specificity poses a huge issue in terms of off-target effects. Dual specificity phosphatases (DSP) such as CDC25 can dephosphorylate a wide range of substrates, including kinases and phosphatases. This makes it challenging to design inhibitors that are specific to a particular DSP, without affecting other enzymes. The more broad-acting DSP would lead to the disruption of Mitogen-activated protein kinase phosphatase, Protein tyrosine phosphatase and Vaccinia H1-related phosphatase activities. Disruption of these DSPs can lead to irregularities in EGFR-initiated cell signalling cascades which could in fact lead to cancers (Wang et al., 2011). The structure of CDC25 as a DSP leads to difficulty in finding new potent inhibitors, arising from three obstacles presented by the active site on the Cdc25 phosphatase: the shallow active site region, the highly reactive cysteine in the active site, and the lack of homology with other protein phosphatases (Lavecchia et al., 2010).

To date there are not many CDC25 inhibitors that have shown significant results in immunosuppressed xenografted mouse models, and of the few I have mentioned the most relevant ones in terms of cancer therapeutics. The most notable to reach phase II of clinical trials was Debio 0931, the licensing name for IRC 083864 in 2009 but there is no news of if they have succeeded to phase III or require more confirmatory results. This reflects the lack of efficacy and displays the lack of translation of the *in-vitro* results to *in-vivo* and may be a significant drawback for using CDC25 inhibitors for cancer therapeutics. The promising technologies of omics and machine learning modules in biological chemistry seem to hold promise for identifying new molecules and derivatives that may be more successful than the current inventory of CDC25 inhibitors. Using some of the more chemically involved algorithms novel and synergetic CDC25 inhibitors have been found that demonstrate inhibition at the protein level (Lauria et al., 2021).

The repertoire of functional cell cycle checkpoint inhibitors is further expanded with the development of chemically inexpensive techniques, namely, click-chemistry, to build off putative backbones that demonstrate functional inhibitory activity. Constructive techniques have proven effective for radioligand imaging for immune checkpoint inhibitors, translation of this development for use in developing in cell cycle check-point inhibitors is not farfetched as reported in non-small cell lung cancer (Vartak et al., 2023). The power of which is expanded through integration of such formulation techniques with chemical library searching (Kabakci et al., 2019) prioritizing scaffolds, such as the quinone backbone, that show functional cell cycle inhibition *in-vitro* demonstrated in NSC663284 in colorectal cancer (Narwanti et al., 2023). The *in-vitro* testing is crucial as it can and has been used to validate *in silico* modelled compounds in other case types for anti-cancer effects (Berlow et al., 2019), which may slow down discovery but increases

validity when combined with genes or gene products identified from omics techniques that are subsequently modelled for inhibitors (Chua et al., 2021; Wu Y. et al., 2024).

9.2 Exploring combination therapies involving CDC25 inhibitors

There are a growing number of studies that present personalized treatment with combination therapies improving outcomes in patients with refractory malignancies (Sicklick et al., 2019). Multimodal therapies have been observed to stratify risk, have stronger chemotherapy, and multimodality treatment strategies have significantly improved the mortality for children with cancer. Initial *in vitro* results showed additive and synergistic effects with other cell cycle inhibitors indirectly, or directly in the CDC25-specific inhibition paradigm that proved promising in their anti-proliferative action on various cancer cell lines (Larsson et al., 2009; Ock and Kim, 2021; Zhao et al., 2023). However, more improvement in survival rates and a decrease in long-term negative effects are required (Forrest et al., 2018). Many studies are coming to the same conclusion that combinatorial methods encompassing genomics, transcriptomics, and pathological images have improved prognostic models for various cancer types (Shao et al., 2023).

These methods have been involved with biomarkers not too dissimilar to the cell cycle marker CDC25 in cancers affecting large swathes of the population, including oesophageal squamous cell carcinoma (Liu et al., 2023), breast cancer (Greenwalt et al., 2020) and colorectal and bladder cancers (Ciardiello et al., 2022; Kong et al., 2022) and gastric cancers (Lee et al., 2019). Growing numbers of studies have pushed this field of study into clinal trials with promising results, as was seen with the gastric cancer umbrella study and in subsets of larger precision oncology clinical trials (O'Dwyer et al., 2023; Rodon et al., 2019) not only showing whether a particular clinical trial for a therapy is effective but by virtue of testing increasing knowledge of molecular significance of tumors; HER2 amplification for instance was found at a frequency of 2% in multiple tumors, not including breast cancers and gastric cancers (Jhaveri et al., 2019). However, analysis from other trials, in which specific biomarkers like CDKN2A, KRAS, and PIK3CA were identified, has shown that these methods may still provide limited results and require further development before entering regular patient processing procedures (Trédan et al., 2019). Clinical trials may seem to lag on the potential of personalizing treatments with regard to aberrant cell cycle control checkpoints.

The synergy between click chemistry methodologies and the conjugation of small molecule inhibitors to monoclonal antibodies represents an innovative Frontier in personalized cancer therapy within the realm of precision medicine. Click chemistry's precision in molecular design and modification allows for the customization of small molecule inhibitors, enabling their conjugation to monoclonal antibodies tailored to individual patients' molecular profiles. Additionally, the involvement of mathematical modelling as well as machine learning modalities can expand potential useful conjugates (Pang et al., 2023). This personalized and *in silico* refined approach facilitates the development of targeted therapeutic agents designed to recognize and bind with high specificity to unique antigens or surface markers present on an individual's cancer cells (Vartak et al., 2023; Greenlee et al., 2024). By leveraging the specificity of monoclonal antibodies for these patient-specific molecular signatures, click chemistry empowers the creation of highly personalized multifunctional agents capable of precisely delivering the small molecule inhibitors to the patient's specific cancerous lesions. This personalized targeting strategy holds immense promise in tailored cancer therapy within the framework of precision medicine, offering a bespoke and targeted therapeutic avenue by harnessing the amalgamation of small molecule inhibitors, monoclonal antibodies, and individualized molecular characteristics for enhanced treatment outcomes.

Personalized medicine data along with advanced chemical-focused exploratory algorithms (Rifaioglu et al., 2019) can direct combination therapies based on novel assay methods working through databases in a high-throughput manner (Lauria et al., 2021). Artificial intelligence along with traditional methods of immunotherapy and chemotherapy have preliminarily shown beneficial prognosis for cancer patients based on results from *in-vitro* assays and limited clinical trials (Kuenzi et al., 2020). Identifying and typing cancerous tissue from benign tissue have been investigated more than the clinical trials, AI models have been developed to predict RNA-Seq profiles and MSI in various cancer types, incorporating multimodal data and transfer learning for improved predictions in various cancers including breast, gastrointestinal and colorectal cancer types (Shao et al., 2023). In endometrial cancer a variety of combinatorial treatments were tested in clinical trials and applied based on molecular markers found within patients' malignant tissue, increasing late-stage patients from 40% to almost 80% survival after more precise (Arend et al., 2018). Combining molecular omics and radiomics (Avanzo et al., 2020) can potentially increase the effectiveness of targeted radiotherapy by reducing and eliminating patients that would prove unsuccessful or remittent to such an approach.

10 Conclusion

Ultimately the evidence reveals that CDC25 inhibitors have tremendous potential as a therapeutic target for cancer therapy, but we would not recommend it as complete but may be part of a greater whole to additionally target in combinatorial therapy. These inhibitors, by modulating CDC25 activity, can impede CDK dephosphorylation and subsequent cell cycle progression, resulting in cell cycle arrest, apoptosis, and decreased tumour development. Preclinical investigations using numerous CDC25 inhibitors, including small molecules, peptides, and natural substances, have yielded encouraging findings, exhibiting significant antitumor action in several cancer types. Clinical trials examining the safety and effectiveness of these inhibitors as monotherapy or in combination with other anticancer treatments, however, are still in the early stages and should validate the results found in the many *in-vitro* studies. Furthermore, the possible toxicity and off-target consequences of these inhibitors should be explored further. The combinatorial treatment high throughput information driven paradigm alongside comprehensive CDC25 inhibitors are some of the most promising directions in halting cell cycle progression in cancer and should garner more focus with regards to clinical trials, even though they may be more difficult to design. While the development of

CDC25 inhibitors as a cancer therapy method is still in its early stages and has faced some obstacles, data shows that this technique has tremendous potential and needs further exploration.

Author contributions

ID: Writing–original draft. AH: Investigation, Writing–review and editing. EA-G: Investigation, Writing–review and editing. WE-H: Writing–review and editing, Funding acquisition. JT: Writing–review and editing, Conceptualization, Investigation. RH: Investigation, Writing–review and editing. MS: Investigation, Writing–review and editing. YB: Writing–review and editing, Conceptualization, Funding acquisition.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was supported by University of Sharjah (Research Project No. (23010901139).

Acknowledgments

The authors would like to express their gratitude to the University of Sharjah by the University of Jordan and Al-Ahliyya Amman University for their continuous support and for supplying the necessary resources for this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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TYPE Mini Review
PUBLISHED 24 January 2024
DOI 10.3389/fphar.2024.1328460

OPEN ACCESS

EDITED BY
Mamoun Ahram,
The University of Jordan, Jordan

REVIEWED BY
Sadanand Pandey,
Yeungnam University, Republic of Korea
Zuhong He,
Wuhan University, China

*CORRESPONDENCE
Elisa Tavazzani,
elisa.tavazzani@unipv.it
Paolo Spaiardi,
paolo.spaiardi@unipv.it

†These authors have contributed equally to this work and share first authorship

‡These authors share senior authorship

RECEIVED 26 October 2023
ACCEPTED 08 January 2024
PUBLISHED 24 January 2024

CITATION
Tavazzani E, Spaiardi P, Contini D, Sancini G, Russo G and Masetto S (2024), Precision medicine: a new era for inner ear diseases. *Front. Pharmacol.* 15:1328460. doi: 10.3389/fphar.2024.1328460

Precision medicine: a new era for inner ear diseases

Elisa Tavazzani[1,2*†], Paolo Spaiardi[3,4*†], Donatella Contini[5], Giulio Sancini[6,7‡], Giancarlo Russo[8‡] and Sergio Masetto[8‡]

[1]Department of Molecular Medicine, University of Pavia, Pavia, Italy, [2]ICS-Maugeri IRCCS, Pavia, Italy, [3]Department of Biology and Biotechnology "Lazzaro Spallanzani", University of Pavia, Pavia, Italy, [4]Istituto Nazionale di Fisica Nucleare, Sezione di Pavia, Pavia, Italy, [5]Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL, United States, [6]Department of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy, [7]Nanomedicine Center, Neuroscience Center, School of Medicine and Surgery, University of Milano-Bicocca, Milan, Italy, [8]Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy

The inner ear is the organ responsible for hearing and balance. Inner ear dysfunction can be the result of infection, trauma, ototoxic drugs, genetic mutation or predisposition. Often, like for Ménière disease, the cause is unknown. Due to the complex access to the inner ear as a fluid-filled cavity within the temporal bone of the skull, effective diagnosis of inner ear pathologies and targeted drug delivery pose significant challenges. Samples of inner ear fluids can only be collected during surgery because the available procedures damage the tiny and fragile structures of the inner ear. Concerning drug administration, the final dose, kinetics, and targets cannot be controlled. Overcoming these limitations is crucial for successful inner ear precision medicine. Recently, notable advancements in microneedle technologies offer the potential for safe sampling of inner ear fluids and local treatment. Ultrasharp microneedles can reach the inner ear fluids with minimal damage to the organ, collect µl amounts of perilymph, and deliver therapeutic agents *in loco*. This review highlights the potential of ultrasharp microneedles, combined with nano vectors and gene therapy, to effectively treat inner ear diseases of different etiology on an individual basis. Though further research is necessary to translate these innovative approaches into clinical practice, these technologies may represent a true breakthrough in the clinical approach to inner ear diseases, ushering in a new era of personalized medicine.

KEYWORDS

vestibule, cochlea, inner ear, nanoparticles, microneedles, precision medicine

1 Background

The ear is anatomically subdivided into three parts: outer, middle, and inner ear (Figure 1). The outer part includes the auricle and the external auditory canal, and its function is to convey the acoustic vibrations to the 105 µm-thick tympanic membrane (Hentzer, 1969; Hayes et al., 2013; Dsouza et al., 2018). The middle ear transmits the vibration of the tympanic membrane to the oval window membrane of the inner ear via the malleus, incus and stapes. Like the outer ear, the middle ear contains air since it is connected to the nasopharynx by the Eustachian tube. In contrast, the inner ear is filled with fluids and houses the cochlea, the auditory organ, and the vestibular system, the equilibrium organ. Within the cochlea, the *scala tympani* and *scala vestibuli* are connected to each other through the helicotrema at the apex of the cochlea and are filled with perilymph, a standard Na^+-based extracellular solution; the *scala media*, or the middle chamber contains

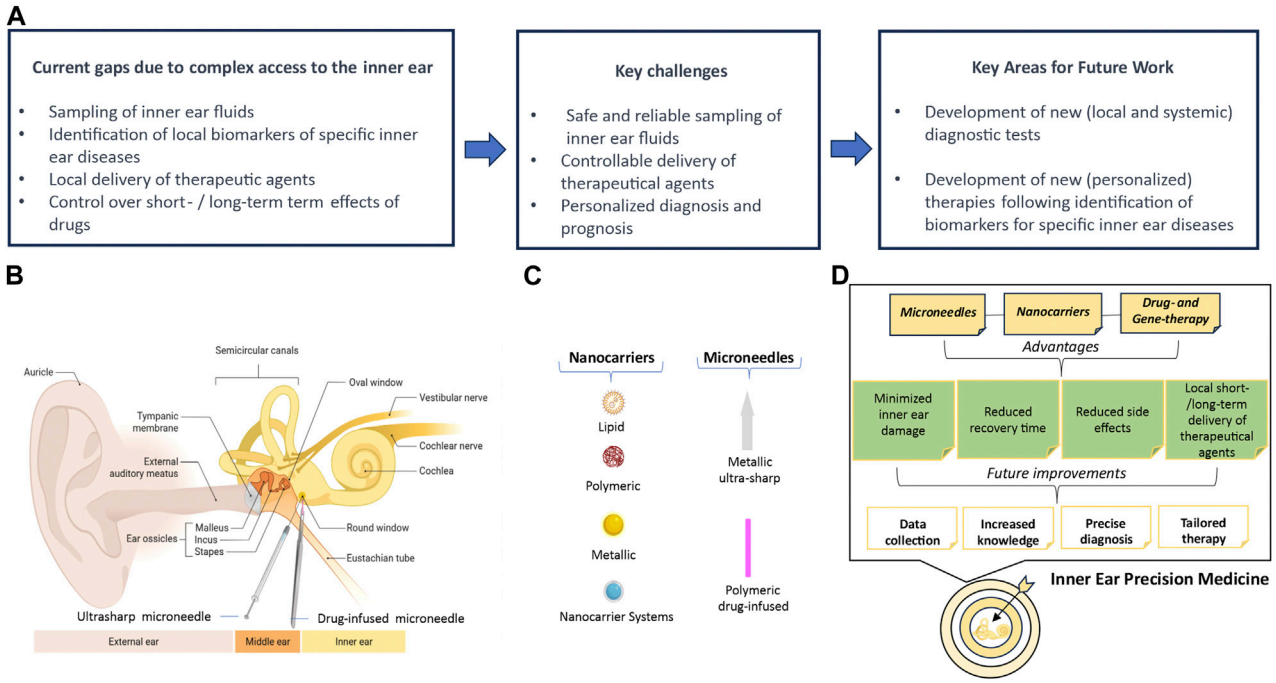

FIGURE 1
Essential features for precision medicine in inner ear. **(A)** Current gaps, goals to reach in the near future, and future aims. **(B)** Schematic drawing of the human ear showing the RWM approach for two types of microneedles (ultrasharp and PLGA). The RWM separates the middle ear from the inner ear. The inner ear contains the cochlea, the auditory organ, and the vestibular system, the balance organ. This is composed of the utricle and saccule, which detect head position and linear acceleration, and the three semicircular canals, which detect head angular accelerations. **(C)** The most common types of nanocarriers and microneedles that can be used for drug delivery in the inner ear. **(D)** Road map to precision medicine in the inner ear. The panels **B** and **C** were created with BioRender 2024. Licence numbers: TP26BU1SAY, SR26BU1Z32.

endolymph, a unique extracellular solution characterized by high K^+ and low Ca^{2+} and Na^+ concentration (Wangemann et al., 2017). The *scala media* is connected to the vestibular system via the *ductus reuniens*. Additionally, near the round window membrane (RWM), the *scala tympani* is connected to the cochlear aqueduct, which is connected to the subarachnoid space filled with cerebrospinal fluid. The sensory function in the inner ear is carried out by sophisticated sensory cells: the hair cells (HCs), which are equipped with a tuft of long microvilli at their apical surface, called stereocilia. The apical region of the HCs is bathed by the endolymph, while their basolateral region is bathed by the perilymph. Endolymph motion induced by acoustic vibrations or head movements deflects the stereocilia of cochlear or vestibular HCs, thereby opening mechanosensitive cation channels. The resulting depolarization opens voltage-gated Ca^{2+} and K^+ channels expressed in the HC basolateral membrane. Ca^{2+} inflow triggers glutamate exocytosis onto the afferent nerve fibers, while K^+ outflow repolarizes the HC. Signals are then transmitted by the primary neurons to the central nervous system for processing.

Inner ear disorders are the most common sensory pathologies worldwide and include deafness, sensorineural hearing loss, Ménière's disease, benign paroxysmal positional vertigo, labyrinthitis, secondary endolymphatic hydrops, and perilymphatic fistula. The World Health Organization estimates that 5.5% of the world's population experiences moderate or high hearing loss and that by 2050, 1 in 10 people will have a disabling hearing deficit (World Health Organization, 2021). Additionally, the incidence of balance disorders of peripheral vestibular origin increases after the age of 40 and exceeds 80% of people over 80 years of age (Agrawal et al., 2009). Ménière's disease alone affects 2 over 1,000 people (Alexander and Harris, 2010; Koenen and Andaloro, 2021). This pathology causes devastating vertigo attacks (Yardley et al., 2003) and standard treatment with gentamicin leaves patients with permanent cochlear deficits (Blakley, 2000; Harner et al., 2001). Impairment of inner ear function severely affects work and social life (Quaranta et al., 2015). The importance of auditory and vestibular functions in daily activities, the widespread incidence of hearing loss and vestibular dysfunction, and the lack of selective inner ear sampling for accurate diagnosis and therapies provide a strong clinical and ethical motivation to expand the research in this field.

2 Major gaps in diagnosis and therapy of inner ear diseases

The location of the inner ear in the osseous labyrinth, the densest and hardest human bone (Frisch et al., 1998), greatly complicates perilymph sampling, which is required for a timely and precise diagnosis, and local drug delivery.

When a patient has an acute or chronic illness of the inner ear, it is likely to be reflected by abnormalities in the presence or concentration of various ions, proteins, bacteria, or viruses when compared to healthy perilymph. Clinically, this limitation results in

a frequent diagnosis of idiopathic hearing/vestibular disease, leaving the patient and physician with no clear prognosis and general, and often ineffective, treatment options.

Current procedures for inner ear fluid sampling use glass capillaries, which do not perforate the RWM and therefore can only be used in patients already under surgery, e.g., for removal of acoustic neuromas or cochlear implantation (Lysaght et al., 2011).

Concerning treatment, the main routes of drug administration to the inner ear are oral, intravenous, intracochlear and intratympanic.

Oral is the simplest way, while intravenous requires medical staff. Both allow for only small lipophilic drugs to be administered because the blood-labyrinth barrier precludes the passage of high-molecular-weight drugs. As a result, only a very low fraction reaches the inner ear (Parnes et al., 1999; Bird et al., 2007). Therefore, either a relatively large amount of drug is needed, which increases the importance of side effects, or the treatment is only minimally effective (Nakashima et al., 2003; Ishiyama et al., 2017).

Intracochlear administration is used in preclinical studies to test the safety of drug candidates and has been adapted to different formulations: liquid, suspension, gel (De Ceulaer et al., 2003; Paasche et al., 2006; Braun et al., 2011). It has the advantage of achieving long-term local drug delivery (weeks or even years). The drawback is the low injectable volume, few μL (Braun et al., 2011), because even slight volume and pressure changes can harm the fragile inner ear organs (Jaudoin et al., 2021). Moreover, it requires surgery under general anesthesia performed by highly specialized personnel (El Kechai et al., 2015a). For these reasons, the intracochlear route is not generally used in humans.

The fourth route, intratympanic administration, is widely used for pharmacological treatment in Ménière's disease (Patel, 2017) due to its minimal invasiveness and compatibility with repeated injections of liquid, suspension, or hydrogel formulations. Intratympanic administration uses a long and fine needle, spinal puncture needles from 22 to 25 G in human (Herraiz et al., 2010) and from 27 to 30 G in animals (Wang et al., 2009; Salt et al., 2011). Then, a volume of 0.4–0.6 mL in humans (Herraiz et al., 2010) or 50–200 μL in animals (Wang et al., 2009) of drug solution is injected, filling the middle ear cavity and ensuring that the drug is in contact with the RWM (El Kechai et al., 2015b). One problem with this approach is that the hole produced in the tympanic membrane is a potential entry point for pathogens. The drug is absorbed into the inner ear perilymph primarily through the semi-permeable RWM, but also via the oval window annular (Phillips and Westerberg, 2011). Since the injected drug must diffuse through the middle ear to reach the RWM, some loss of the medicine occurs through the Eustachian tube (Ramaswamy et al., 2017), resulting in highly variable medication levels among patients. Direct placement of therapeutic agents on the RWM in a biodegradable carrier substance, such as gelatin, hydrogel, or nanoparticles, may overcome some of these limitations (Paulson et al., 2008; Li et al., 2012; Zhang et al., 2018). However, the rate of drug delivery to the inner ear is inevitably limited by molecular diffusion across the RWM. In summary, the reliability of intratympanic administration is severely impacted by the variable diffusion of the drug through the middle ear and the RWM.

Given the above, it is evident the need for safe and reliable access to the inner ear for both diagnosis and treatment purposes.

3 Microneedles may help filling the gaps

Without a means to sample inner ear fluid for electrochemical, genetic, or proteomic analysis, precise intervention is not possible. Furthermore, current options for drug delivery, including systemic administration and intratympanic injection, are imprecise. The only access from the middle ear to the inner ear that does not require perforation of bone is through the RWM. The human RWM has a surface area of 2.3 mm^2 (Okuno and Sando, 1988) and a thickness of 70 μm (Goycoolea and Lundman, 1997). The distance between the human RWM and the basilar membrane—the nearest structure within the inner ear—is approximately 1.2 mm (Paprocki et al., 2004; Watanabe et al., 2016). The primary risks associated with RWM perforation concern damage to the fragile structures of the inner ear, homeostasis impairment due to perilymph leakage and external contamination. Fortunately, recent technical advances in microneedle architecture and manufacturing have minimized the negative consequences of RWM perforation, also reviewed in Leong et al. (2022). Chiang et al. (2020), manufactured 3D-printed microneedles able to create small (μm scale) holes in cadaveric human RWM without damaging the cochlea. 3D printing relies on a technology called two-photon polymerization lithography, an additive manufacturing process that can produce highly complex geometries out of hard polymers with sub-micrometer precision and accuracy. Fully-metallic (copper) ultra-sharp microneedles were conversely developed by Aksit and colleagues (Aksit et al., 2021), which were gold-coated to ensure biocompatibility. Minimal trauma was observed in both guinea pig and human cadaveric RWMs. Thus, microneedle RWM perforation might be performed prior to intratympanic delivery to overcome variable diffusion of the therapeutic agent through the RWM (Szeto et al., 2019). Concerning perilymph sampling, Early and colleagues (Early et al., 2019) developed a silver-plated hollow microneedle device for trans-RWM liquid biopsy, by which 1 μL of perilymph from post-mortem human temporal bones could be obtained without damaging the inner ear structures. This is a notable result when considering that the whole perilymph per ear is about 150 μL (Leong et al., 2023a). By the same strategy, Leong and colleagues (Leong et al., 2023a) very recently developed a 3D-printed hollow microneedle for diagnostic aspiration of perilymph and intracochlear delivery of therapeutic agents in living guinea pigs. Perforation did not cause hearing loss, healed within 48–72 h, and yielded sufficient perilymph for proteomic analysis. A slightly different approach has been used by Pawley and colleagues (Pawley et al., 2021), who developed a drug-infused polymeric microneedle designed for penetrating the RWM and resting in the base of the scala tympany of the rat cochlea, to deliver a uniform dose of the drug over an extended period (about 50% of drug was released in 1 month). Microneedles were made of poly (lactic-co-glycolic acid); (PLGA), a biodegradable polymeric nanoparticle (see next section) approved by the Food and Drug Administration. Their microneedles had over ~4,000× the mechanical strength required to puncture rodent RWM and are therefore suitable for human use. This is the only study reporting delivery of therapeutic agents by microneedles so far.

In summary, microneedle-mediated perforation of the RWM is a novel means of achieving access to the inner ear with minimal

TABLE 1 Main properties of microneedles.

Microneedles' properties
1. The materials used for the fabrication (metals, polymers) are hard and resistant enough to perforate the RWM in both animal models and humans (Early et al., 2019; Szeto et al., 2019; Chiang et al., 2020; Yu et al., 2020; Aksit et al., 2021; Pawley et al., 2021)
2. The manufacturing techniques are highly versatile, allowing for different shapes and diameters of the microneedle tips (Watanabe et al., 2016; Early et al., 2019; Szeto et al., 2019; Yu et al., 2020; Aksit et al., 2021; Leong et al., 2022)
3. The same microneedle can be luminized and used to perforate the RWM and sample or/and deliver therapeutic agents, streamlining the procedure. This eliminates the need for the double procedure involving RWM perforation first, and sampling/delivery by a second device (Szeto et al., 2019; Chiang et al., 2020; Yu et al., 2020; Aksit et al., 2021; Pawley et al., 2021; Leong et al., 2022)
4. The materials employed for the microneedles are biocompatible (Aksit et al., 2021; Pawley et al., 2021; Leong et al., 2023b)
5. The architecture enables control of perforation by detecting the reaching of the perilymph (Wazen et al., 2017; Early et al., 2019)
6. The tip of the microneedles can be designed to smoothly cross the RWM, minimizing damage (reducing healing time) and perilymph leakage in the middle ear (Early et al., 2019; Aksit et al., 2021; Leong et al., 2023a; Feng et al., 2023)
7. The new microneedles can be used for sampling or acute delivering (ultra-sharp microneedle) or implanted for long-term delivery (PLGA microneedles) due to their biodegradable nature (Aksit et al., 2021; Pawley et al., 2021; Leong et al., 2023b; Feng et al., 2023)
8. The tiny dimensions of the microneedles tips allow for sampling/delivering small (μl scale) volumes (Aksit et al., 2021; Leong et al., 2023a; Feng et al., 2023)

anatomic and functional damage. Microneedles are designed to aspirate fluids for diagnosis and to deliver therapeutic agents. Using microneedles, drug concentrations within the inner ear may be controlled with a precision that intratympanic injections cannot provide. Microneedle's properties are summarized in Table 1. *In vivo*, microneedles have only been tested in rodents so far, although perforation of human RWM has been accomplished *postmortem*. It should be noted that RWM perforation requires surgery of the temporal bone and therefore cannot be considered as a simple routine procedure.

4 Nanocarriers for drug delivery to the inner ear

The treatment of inner ear disorders may soon benefit from the rapid development of nanocarriers to deliver drugs and genetic material. The obstacles to overcome, besides access to the inner ear as discussed above, are: a) the control for long-term release of the drug; b) the development of side effects; c) the degradation of the carrier/carried agent. The biocompatibility, biodegradability, size, volume, composition, shape and chargeable surface of nanocarriers, their bio-distribution in the target and off-target organs, metabolism and elimination are all important features to be addressed.

The main nanoparticles (NPs) so far considered for inner ear therapy are listed below, categorized for their molecular nature (Figure 1C):

A) Lipid-Based Nanocarriers: Liposomes, Solid Lipid Nanoparticles (SLNs), Nanostructured Lipid Carriers (NLCs).

Liposomes, the first phospholipid vesicle system developed in the 1960s, are composed of phospholipid bilayer similar to the plasma membrane of human cells. They have good biocompatibility and promote drug diffusion across the plasma membrane. Liposomes, with sizes ranging from 20 nm to over 1 μm, typically consist of a hydrophobic bilayer that encapsulates lipophilic drugs and a hydrophilic core for holding and stabilizing hydrophilic drugs. Such features make liposomes a versatile vehicle to carry both hydrophilic and hydrophobic drugs in the aqueous lumen and lipid bilayer respectively. In addition, the liposome system offers the advantage of easy modification and targeting potential. Liposomes can be constructed with their surfaces modified using appropriate molecules or ligands to actively bind a target molecule within specific cells, systems, or tissues (Lu et al., 2021). Recently, liposomes have been used to treat autosomal dominant hearing loss by *in vivo* delivery, through a cannula following cochleostomy, of genome editing agents in transgenic mice (Gao et al., 2018).

SLNs combine the advantages of polymer nanocarriers, including a high drug loading capacity, controllable drug delivery, and good biocompatibility with lipid emulsions, thereby improving drug bioavailability. SLNs can be prepared by using a variety of technologies including heat or cold homogenization, making them easily scalable for production. Due to their small sizes and large surface area, SLNs are suitable to be covered with functionalized ligands moieties, antibodies, and other functional groups (Lu et al., 2021).

NLCs, also known as lipid-based formulations, have been broadly studied as drug delivery systems due to their enhanced physical stability, improved drug loading capacity, and biocompatibility (Haider et al., 2020). Unlike SLNs, the lipid matrix of NLCs consists of a mixture of solid and liquid lipids with controlled levels that have an improved capacity for bioactive retention along with controlled release attributes (Akhavan et al., 2018).

B) Polymeric Nanoparticles: Polymersomes may pass the RWM without toxic effects (Buckiova et al., 2012; Roy et al., 2012); Chitosan is a natural biodegradable and biocompatible polymer with antifungal and antibacterial properties deliverable in the inner ear (No et al., 2002; Saber et al., 2010; Lajud et al., 2015; Khanna et al., 2023; Rezaei Abbas Abad et al., 2023); PLGA NPs are suitable for the treatments of inner ear diseases for their tunable degradation, mechanical properties, drug-infused microneedle fabrication (Lehner

et al., 2019; Pawley et al., 2021), and gene therapy (Du et al., 2018).

C) Metallic NPs: Superparamagnetic Iron Oxide Nanoparticles permit a precise drug delivery in the inner ear by magnetic forces (Kopke et al., 2006); Silver Nanoparticles have antifungal, antibacterial, antiviral properties and are suitable in otitis treatment (Zou et al., 2014); Gold Nanoparticles are chemically stable and biocompatible (Blebea et al., 2022); Silica Nanoparticles may serve as a nonviral delivery system to the sensory HCs (Praetorius et al., 2007).

D) Nanocarrier Systems: Nanoparticle-Hydrogel Systems are thermosensitive solidifying in the middle ear for a sustained dose release (Lambert et al., 2016); Cell Penetrating Peptides can be used to deliver cargo into the developing inner ear (Miwa et al., 2011).

For comprehensive reviews classifying nanocarrier biodistribution, pathway mechanisms and drug pharmacokinetics see: Jaudoin et al., 2021; Dindelegan et al., 2022.

5 Inner ear and gene therapy

Thanks to recent advances in understanding the genetic basis of several inner ear diseases, delivery of genetic material (DNA, RNA, siRNA, microRNA, antisense oligonucleotides, or CRISPR/Cas9) has emerged as a promising strategy for their treatment (Lentz et al., 2020; Bankoti et al., 2021; Cui et al., 2022; Nacher-Soler et al., 2022). This approach aims to control gene replacement, silencing, augmentation, and editing and it could be an option to treat hearing loss and vestibular disorders (Fukui and Raphael, 2013; Geng et al., 2018; Lentz et al., 2020; Bankoti et al., 2021; Cui et al., 2022; Nacher-Soler et al., 2022). The most common and successful way of delivering genetic material to the inner ear of rodents is through the RWM (Akil et al., 2012; Askew et al., 2015; Pan et al., 2017a; Emptoz et al., 2017; Landegger et al., 2017; Dulon et al., 2018) further highlighting the importance of perfecting this route of administration for future use in humans. Notably, György and colleagues (Gyorgy et al., 2019) recently showed that the RWM approach leads to efficient transgene transfer into the cochlea of non-human primates (Gyorgy et al., 2019). Alternative delivery routes to the inner ear, so far positively tested in rodents, involve injection of agents into the posterior semicircular canal (Okada et al., 2012; Suzuki et al., 2017; Isgrig and Chien, 2018), or into the cerebrospinal fluid of the *cisterna magna*, which is connected to the inner ear by the cochlear aqueduct (Mathiesen et al., 2023). The combination of trans-RWM injection and canalostomy in adult mice has recently been shown to increase the efficiency of gene transduction in cochlear inner HCs in all turns of the cochlea without impairing auditory function or hearing (Yoshimura et al., 2018).

However, several questions need to be answered before gene therapy translation to humans. Due to challenges in maintaining inner ear tissues *in vitro*, tests in human tissues are still limited (Kesser et al., 2007). Unresolved points include differences in cell trophism and chronological maturation between humans and animal models, and strategies to confine gene therapy to target organs. Addressing issues such as negative consequences of gene overexpression/silencing in target and off-target cells, as well as evaluating the long-term safety of exogenous constructs, immune response in case of multiple administrations, time between doses, and risk of infection before and after gene therapy is imperative (Wu et al., 2019).

Delivery routes and vectors depend on material size, cargo capacity, pathogenicity, immunogenicity, and transduction efficiency. There are two main delivery systems for gene therapy: (i) viral vectors: viruses modified and attenuated to create effective and specific tools for gene transfer, and (ii) non-viral delivery/vectors: nanoparticles and microspheres consisting of biodegradable polymers. Adeno-associated viral vectors (AAVs) and synthetic viral vectors (Anc80L65 and AAV2.7m8) are commonly chosen for their effectiveness in the inner ear (Pan et al., 2017b; Isgrig et al., 2017). Non-viral vectors (nanoligand drug carriers self-assembled from a phage display peptide) (Delmaghani and El-Amraoui, 2020) avoiding virus integration into human DNA, represent alternatives due to their easy use, reduced toxicity and immunogenicity compared to viral vectors. Careful consideration of these factors is essential for successful gene therapy applications. For extensive reviews about gene therapy in the inner ear see Delmaghani and El-Amraoui and Chaves & Holt (Delmaghani and El-Amraoui, 2020; Chaves and Holt, 2023). Gene therapy could also take advantage of microneedles for delivery.

6 Discussion and future prospects

One of the critical determinants of the success of precision medicine in the inner ear is to find safe and reliable access to perilymph for personalized diagnosis and delivery of pharmacological agents. The new ultrasharp microneedles meet these requirements, and together with nano vectors and gene therapy offer great promise as a potential treatment for human inner ear disease of environmental or genetic cause. In perspective, sampling of inner ear fluids could identify biomarkers of specific diseases that might be also detected in routine blood test (Mahshid et al., 2022), providing crucial information for therapy and assessment of treatment efficacy. Safe delivery to the inner ear, moreover, might allow the use of contrast agents for precise visualization of inner ear disorder (e.g., endolymphatic hydrops) by imaging. Guidelines will be necessary to establish shared approaches concerning, for example, the optimal volume for sampling/injection, the type of microneedle for a given treatment, the follow-up. Strong collaborative efforts are required between researchers, clinicians, companies, and regulatory agencies to unlock the great potential of precision medicine in the inner ear.

Author contributions

ET: Conceptualization, Supervision, Writing–original draft, Writing–review and editing. PS: Conceptualization, Funding acquisition, Supervision, Writing–original draft, Writing–review and

editing. DC: Conceptualization, Supervision, Writing–original draft, Writing–review and editing. GS: Conceptualization, Funding acquisition, Investigation, Supervision, Writing–original draft, Writing–review and editing. GR: Conceptualization, Supervision, Writing–original draft, Writing–review and editing. SM: Conceptualization, Funding acquisition, Supervision, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The fee of this publication is supported by the following grants: FIS. SPAIA.PRIN 2022; PRIN 2022 funded by the European Union–Next-Generation EU, CUP F53D23006030001; PRIN 20228AAJRL; PRIN 2022 funded by the European Union–Next-Generation EU, CUP F53D23005930006; 2023-ATEQC-0026—multifunctional nanocarriers as advanced biomaterials for targeted and therapeutic RNA delivery [MixnMatch] financed to: SP, MS, and SG respectively.

Acknowledgments

Figure 1 (B and C panels) was adapted from "Middle and Inner Ear Anatomy", and "Icon Pack–Nanoparticles" by BioRender.com (2023). Retrieved from https://app.biorender.com/biorender-templates.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Pharmacology

TYPE Original Research
PUBLISHED 05 February 2024
DOI 10.3389/fphar.2024.1309766

OPEN ACCESS

EDITED BY
Mamoun Ahram,
The University of Jordan, Jordan

REVIEWED BY
Beatrice Polini,
University of Pisa, Italy
Oriana Awwad,
The University of Jordan, Jordan

*CORRESPONDENCE
Emanuela Martino,
emanuela.martino@unipv.it
Simona Collina,
simona.collina@unipv.it

RECEIVED 08 October 2023
ACCEPTED 19 January 2024
PUBLISHED 05 February 2024

CITATION
Cavalloro V, Marchesi N, Linciano P, Rossi D, Campagnoli LIM, Fossati A, Ahmed KM, Malacrida A, Miloso M, Mazzeo G, Abbate S, Longhi G, Ambrosio FA, Costa G, Alcaro S, Pascale A, Martino E and Collina S (2024), Neurodegeneration: can metabolites from *Eremurus persicus* help?
Front. Pharmacol. 15:1309766.
doi: 10.3389/fphar.2024.1309766

Neurodegeneration: can metabolites from *Eremurus persicus* help?

Valeria Cavalloro[1,2], Nicoletta Marchesi[3], Pasquale Linciano[3], Daniela Rossi[3], Lucrezia Irene Maria Campagnoli[3], Alice Fossati[1,2], Karzan Mahmood Ahmed[4], Alessio Malacrida[5,6], Mariarosaria Miloso[5,6], Giuseppe Mazzeo[7], Sergio Abbate[7], Giovanna Longhi[7], Francesca Alessandra Ambrosio[8], Giosuè Costa[9,10], Stefano Alcaro[9,10,11], Alessia Pascale[3], Emanuela Martino[1,2]* and Simona Collina[3]*

[1]Department of Earth and Environmental Sciences, University of Pavia, Pavia, Italy, [2]National Biodiversity Future Center, Palermo, Italy, [3]Department of Drug Sciences, University of Pavia, Pavia, Italy, [4]Department of Chemistry, College of Education, University of Garmian, Kalar, Iraq, [5]School of Medicine and Surgery, University of Milan-Bicocca, Monza, Italy, [6]Experimental Neurology Unit, University of Milano-Bicocca, Monza, Italy, [7]Department of Molecular and Translational Medicine, Università di Brescia, Brescia, Italy, [8]Department of Experimental and Clinical Medicine, University "Magna Græcia" of Catanzaro, Campus "S. Venuta", Catanzaro, Italy, [9]Department of Health Sciences, Campus "S. Venuta", "Magna Græcia" University of Catanzaro, Catanzaro, Italy, [10]Net4Science Academic Spin-Off, University "Magna Græcia" of Catanzaro, Campus "S. Venuta", Catanzaro, Italy, [11]Associazione CRISEA–Centro di Ricerca e Servizi Avanzati per l'Innovazione Rurale, Italy

The number of patients affected by neurodegenerative diseases is increasing worldwide, and no effective treatments have been developed yet. Although precision medicine could represent a powerful tool, it remains a challenge due to the high variability among patients. To identify molecules acting with innovative mechanisms of action, we performed a computational investigation using SAFAN technology, focusing specifically on HuD. This target belongs to the human embryonic lethal abnormal visual-like (ELAV) proteins and plays a key role in neuronal plasticity and differentiation. The results highlighted that the molecule able to bind the selected target was (*R*)-aloesaponol-III-8-methyl ether [(*R*)-ASME], a metabolite extracted from *Eremurus persicus*. Notably, this molecule is a TNF-α inhibitor, a cytokine involved in neuroinflammation. To obtain a suitable amount of (*R*)-ASME to confirm its activity on HuD, we optimized the extraction procedure. Together with ASME, another related metabolite, germichrysone, was isolated. Both ASME and germichrysone underwent biological investigation, but only ASME confirmed its ability to bind HuD. Given the multifactorial nature of neurodegenerative diseases, we decided to investigate ASME as a proteasome activator, being molecules endowed with this kind of activity potentially able to counteract aggregations of dysregulated proteins. ASME was able to activate the considered target both in enzymatic and cellular assays. Therefore, ASME may be considered a promising hit in the fight against neurodegenerative diseases.

KEYWORDS

***Eremurus persicus*, HuD, embryonic lethal abnormal visual-like, proteasome activators, molecular modeling, *(R)*-aloesaponol-III-8-methyl ether, *(R)*-germichrysone, nature-aided drug discovery**

1 Introduction

In recent years, changes in the demographic trend have led to progressive aging of the population worldwide (Klimova and Kuca, 2016). This scenario is associated with a continuous increase in the number of patients affected by neurodegenerative diseases, among which the most widespread are Alzheimer's, Parkinsonism, and amyotrophic lateral sclerosis diseases (Logroscino et al., 2022; Duarte Folle et al., 2023). The treatments available in this field are often disease-specific and involve the use of small molecules, like memantine, carbidopa, or riluzole, or monoclonal antibodies, like aducanumab, but they are always associated with poor efficacy (Lamptey et al., 2022). Despite many efforts in recent years, no effective treatments have been developed, and patients affected by these pathologies still have a poor quality of life (Maresova et al., 2020). In this scenario, precision medicine may be a powerful tool, but this approach is still challenging due to the high variability among patients. Given the multifactorial nature of neurodegenerative disorders, the development of multi-target drugs acting with innovative mechanisms could represent a winning strategy in developing novel effective treatments against such diseases (Kamecki et al., 2021; Pellavio et al., 2021; Linciano et al., 2023).

To identify molecules able to interact with targets potentially involved in neurodegenerative diseases and acting through multiple mechanisms of action, for this work, we used our in-house compound library. Such a collection, which includes both synthetic molecules and secondary metabolites, has been digitized to speed up the drug discovery process. Collected compounds are characterized by significant structural diversity, lead-likeness, and an overall solubility profile to ensure that the resulting hits are worthy of investigation (MedChem Lab, MCL, Department of Drug Sciences, University of Pavia, Pavia, Italy) (Listro et al., 2023; Pagano et al., 2023). Particularly, we focused on two targets, the RNA-binding protein named HuD and the proteasome system since proteasome activators are potentially able to counteract aggregations of dysregulated proteins (Leestemaker et al., 2017).

HuD protein belongs to the human embryonic lethal abnormal visual-like (ELAV) or Hu protein family, the main function of which is to increase the stability and/or the rate of translation of specific mRNAs. Four human ELAV proteins have been characterized: HuR (or HuA), HuB, HuC, and HuD, all sharing a high degree of sequence homology (70%–85%) (Volpe et al., 2020). Due to their ability to act in different contexts, ELAV proteins are considered pleiotropic proteins; however, while HuR is ubiquitous, HuB, HuC, and HuD are specifically expressed in the nervous system, and they are known as neuronal ELAV (nELAV; Vasile et al., 2018; Nishisaka et al., 2023). In particular, HuD, the most studied among the nELAV, has been recognized as a target dysregulated in several neurodegenerative disorders, like Alzheimer's, Parkinsonism, and amyotrophic lateral sclerosis diseases, although only a few small molecules have been identified as binders so far (Ambrosio et al., 2021; Silvestri et al., 2022; Marchesi et al., 2023; Zhang et al., 2023).

Proteasomes are gaining more and more attention in the scientific community as they are responsible for maintaining cellular homeostasis under physiological conditions and modulating protein degradation during the life of the cell. While proteasome inhibitors as anticancer agents are deeply studied and are already in clinical practice, few data are available on proteasome activators. Recent evidence highlighted how enhancing proteasome activity can be an innovative tool in contrasting dysregulated protein aggregations in neurodegenerative disorders. Again, only a few small organic molecules endowed with this kind of activity have been identified, all being active in the mM range and with different mechanisms of action. Specifically, they have been described to act as inhibitors of deubiquitinase or p38 MAPK enzymes, modulators of cAMP- or cGMP-dependent protein kinase, or as direct 20S activators (e.g., SDS) (George and Tepe, 2021).

Due to the high novelty associated to these targets, the development of a new molecular entity able to both bind HuD and activate the proteasome has not been investigated yet. However, considering the multifactorial nature of neurodegenerative diseases, the development of new multi-target drugs could represent a pivotal step ahead in this field. More in detail, HuD and proteasome can be considered to be two orthogonal targets, one involved in differentiation and synaptic plasticity and the other in the degradation of proteins like synuclein or tau proteins.

In this paper, we report for the first time the medicinal chemistry work performed to identify novel dual ligands endowed with an innovative multi-target profile: HuD-RNA interfering compounds and proteasome activators, potentially active against neurodegenerative diseases.

2 Methods

2.1 SAFAN

SAFAN-*in silico* profiling (SAFAN-ISP) exploits a library of fragments and calculates similarity (Tanimoto) by matching atoms to fragments and evaluating common atoms. Experimental data are obtained using a refactored bioactivity database from the ChEMBL31 database. The steps performed by using the SAFAN platform foresee the fragmentation of the input molecule and the comparison of each fragment to the SAFAN-ISP database. Fragments are used to select targets sharing similar fragments with the input compounds and to calculate affinities by combining data concerning different compounds binding the same target. Next, the binding constant on all targets outputted is computed combining the similarities and experimental data using two schemes including chiral fingerprints and two excluding them. Finally, the RepTree algorithm, available from the WEKA open source package, is used to combine all binding constants obtained in the previous steps in a single value that will be the final output.

2.2 Molecular recognition studies

The (*R*)-aloesaponol-III-8-methyl ether [(*R*)-ASME] compound was prepared by means of the LigPrep tool. Hydrogens were added, salts were removed, and ionization states were calculated using an ionizer at pH 7.4 (SchrödingerLLC, 2018).

For the molecular modeling studies on the HuD protein, we started from the crystal structure of HuD and the (A/U)-rich element of c-fos mRNA, deposited in the Protein Data Bank (PDB) with the PDB code 1FXL (Wang and Tanaka Hall, 2001).

TABLE 1 Tested extraction conditions.

	# Cycles (time for each cycle)	T°C	Solvent renewal	Total EtOH volume (ml)
Mac_1	3 (1 h–12 h–1 h)	r.t.	Yes	600
Mac_2	3 (1 h–1 h–1 h)	r.t.	Yes	600
Mac_3	2 (12 h–1 h)	r.t.	Yes	400
Mac_4	2 (12 h–1 h)	60°C	Yes	400
MASE_1	1 (20 min)	120°C	Yes	200
MASE_2	1 (20 min)	60°C	Yes	200
MASE_3	2 (20 min + 20 min)	60°C	Yes	400
MASE_4	2 (5 min +5 min)	60°C	Yes	400
MASE_5	2 (20 min + 20 min)	60°C	No	200

The receptor structure was processed using the Protein Preparation Wizard tool (LLC, 2018) using the same protocol reported in our previous work (Ambrosio et al., 2021).

The docking studies were performed by means of the Glide v. 6.7 SP algorithm, and 10 poses per ligand were generated (Schrödinger, 2018).

For the modeling studies on the proteasome, we used the crystal structure of the human 20S proteasome complex with ixazomib, deposited in the PDB with the PDB code 5LF7 (Schrader et al., 2016). The structure was prepared by means of the Protein Preparation Wizard tool, using the same protocol reported in our previous works (Malacrida et al., 2021; Listro et al., 2022).

In order to identify and characterize the ligand-binding sites and druggability assessment in the α-rings of the 20S proteasome, the SiteMap program v. 4.6 (SiteMap, Schrödinger, 2018) was used.

According to the druggability score and SiteScore, the best SiteMap identified binding site was selected for further docking simulation, carried out with Glide software v. 6.7 by using the SP algorithm (Schrödinger, 2018), and 10 poses per ligand were generated.

2.3 Plant material and extraction procedure

Eremurus persicus (Jaub. & Spach) Boiss. was collected from a mountainous area (Küh-e Golestän, Golpayegan) located 120 km from Isfahan/Iran, at an altitude of 3000–3200 m. The collected plant materials were identified and classified by Dr. Abdulla Sa'ad at the Education Science Department, Faculty of Biology, Salahaddin University, Hawler/Iraq. The voucher specimen (No. 6856) was deposited at Education Salahaddin University Herbarium (ESUH), Hawler/Iraq. Freshly cut roots were dried in a drying room with active ventilation at room temperature (approximately 20°C–22°C) until they showed no further weight loss. The roots were cut into small pieces and ground with a blade mill (A10 IKA-Werke GmbH & Co. Staufen, Germany) to obtain a homogeneous fine powder. The plant material so treated was stored under dark conditions.

E. persicus extracts were prepared following both maceration and microwave-assisted solvent extraction (MASE) procedures. For the second method, a multi-mode microwave apparatus, using a closed-vessel system (MARSX press, CEM Corporation, Matthews, NC, United States), was exploited, setting 800 W as the maximum potency and 5 min as the ramp time. In order to identify the best extraction conditions in terms of (*R*)-ASME yields, the following conditions were applied (Table 1):

After the extraction process, the solvent was evaporated under reduced pressure using a Heidolph Laborota 4000 instrument (Heidolph Instruments GmbH & Co., Schwabach, Germany) and the yield calculated (Table 2). Next, raw extracts were solubilized in water and extracted five times with ethyl acetate (5 × 80 ml). The organic phases so obtained were then washed with water (3 × 50 ml), dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. This fraction was finally chromatographed (mobile phase: 1 hexane:1AcOEt; stationary phase: silica gel 60 particle size 230–400 mesh purchased from Nova Chimica, Cinisello Balsamo, Italy), allowing the isolation of pure (*R*)-ASME and an unknown metabolite. The entire process was followed exploiting analytical thin-layer chromatography (TLC) on silica gel pre-coated glass-backed plates (Fluka Kieselgel 60 F254, Merck, Darmstadt, Germany) using the same MP. The detection was conducted with UV light (254 and 366 nm).

(*R*)-ASME: $[\alpha]_D = -37.9$ (*c* 0.04, $CHCl_3$). ESI-MS: *m/z* 273 $[M + H]^+$. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.01 (brs, 1H), 2.28 (m, 2H), 2.48 (s, 3H), 2.69 (m, 1H), 3.05 (m, 1H), 4.02 (s, 3H), 4.94 (dd, *J* = 5 Hz, 1H), 6.69 (d, *J* = 2 Hz, 1H), 7.06 (s, 1H), and 7.14 (d, *J* = 2 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 203.3 (s), 165.9 (s), 159.5 (s), 142.0 (s), 140.3 (s), 139.9 (s), 119.9 (d), 115.4 (d), 113.8 (s), 109.2 (s), 108.3 (d), 68.0 (d), 56.0 (q), 34.0 (t), 30.7 (t), and 22.1 (q).

The structure of the unknown metabolite was then investigated with nuclear magnetic resonance (on a Bruker Avance III 400 MHz spectrometer, Milan, Italy), mass spectroscopy (MS) techniques, and optical rotation. Finally, it was elucidated as germichrysone. $[\boldsymbol{\alpha}]_D$ = +12; $[\boldsymbol{\alpha}]_{546}$ = +18; $[\boldsymbol{\alpha}]_{436}$ = +108 (c 0.15 g/100 ml, THF). ESI-MS: *m/z* 259.92 $[M + H]^+$.

^{1}H-NMR (400 MHz, MeOD) δ 6.88 (s, 1H), 6.78 (d, J = 2.4 Hz, 1H), 6.75 (dd, J = 2.3, 0.9 Hz, 1H), 4.32 (tt, J = 7.5, 3.7 Hz, 1H), 3.20 (dd, J = 15.6, 3.6 Hz, 1H), 2.97 (ddd, J = 5.1, 4.4, 2.4 Hz, 2H), and 2.73 (ddd, J = 17.2, 7.5, 0.9 Hz, 1H).

^{13}C-NMR (MeOD): δ 203.5 (s), 158.9 (s), 145.1 (s), 140.5 (s), 136.1(s), 118.5(d), 113.0(d), 111.1 (s), 110.3 (s), 66.5 (d), 46.8 (t), 38.9 (t), and 22.2 (q).

TABLE 2 Extraction and pure metabolite yields related to each extraction protocol.

	Extraction yield (%)	(*R*)-ASME yield	((%)*R*)-Germichrysone yield
M (%)ac_1	13.8	0.14	0.011
Mac_2	9.1	0.11	0.033
Mac_3	12.9	0.21	0.051
Mac_4	25.8	0.24	0.062
MASE_1	20.1	0.14	0.044
MASE_2	5.9	0.16	0.060
MASE_3	8.5	0.24	0.081
MASE_4	9.0	0.27	0.075
MASE_5	6.6	0.18	0.028

2.4 Chiroptical methods

VCD-IR: measurements were carried out using a JASCO FVS 6000 FTIR instrument equipped with a ZnSe photo-elastic modulator (PEM), working at 50 kHz modulation, placed past a wire grid linear polarizer and with a lock-in amplifier after detection, and the latter was obtained with an MCT liquid N_2-cooled diode for the regions 850–2000 cm^{-1}. The tetrahydrofuran-d_8 solution of germichrysone with 0.1 M concentration was recorded in a 200 mm BaF_2 cell. A total of 5,000 scans were acquired, and a similar spectrum was taken for the solvent and subtracted out. **ORD**: measurements were performed with the use of a JASCO 815SE instrument with samples dissolved in tetrahydrofuran at 0.001 M concentration in 5-mm and 0.1-mm quartz cuvettes, in order to record the 350–500 nm and 200–350 nm ranges, respectively. A total of 10 scans per spectra were acquired. The ECD-UV spectra of the solvent were also recorded under the same conditions and subtracted thereafter from the sample ECD spectra. The UV spectra were obtained from the same apparatus. **ORD**: measurements were carried out with a JASCO P-2000 polarimeter. The tetrahydrofuran solution of germichrysone (0.15 g/100 ml) was recorded at three different wavelengths, 589 nm (Na lamp), 546 nm, and 435 nm (Hg lamp), with 10 measurements at each wavelength. **CPL**: the CPL and fluorescence spectra were recorded on a home-built apparatus (Castiglioni et al., 2010) in a 2-mm cell at the same concentration used for ECD-UV measurements. A total of 20 accumulation scans were employed. The excitation wavelength of 375 nm was obtained by using the commercial FP-8200 JASCO fluorimeter and brought to the cell through a water-filled optical fiber. **Calculations**: Density functional theory (DFT) and time-dependent density functional theory (TD-DFT) calculations were performed by use of the Gaussian16 program (Frisch et al., 2016) preceded by a conformational search of all possible conformers by the CREST program (Pracht et al., 2020) assuming (*R*)-configuration ((*R*)-AC) in setting up all calculations (Supplementary Material for details). The B3LYP/TZVP level was employed for DFT (optimizations and VCD-IR calculations), CAM-B3LYP/TZVP for ECD, and CAM-B3LYP/6-311++G (d,p) for ORD, which were used for TD-DFT calculations, both *in vacuo* and in PCM approximation with tetrahydrofuran as the solvent.

TABLE 3 Gradient elution of the developed HPLC method.

Time (min)	A (%)	B (%)
0	50	50
10	30	70
11	50	50
14	50	50

2.5 HPLC

HPLC-grade solvents were supplied by Honeywell (Seelze, Germany). The chromatography procedure was performed using a Thermo Finnigan (Japan) high-performance liquid chromatography–photodiode array system (HPLC-UV/PAD) equipped with a Surveyor Autosampler, a Surveyor Pump, and a Surveyor PDA Plus Detector. Experimental data were acquired and interpreted exploiting Xcalibur software. The stationary phase was represented by a reverse-phase column (Chromolith SpeedROD RP-18 endcapped column; 50 mm × 4.6 mm, ID 3 mm, macropore size 2 μm, mesopore size 13 nm, Merck, Darmstadt, Germany), while the mobile phase was water +0.1% HCOOH as solvent A and MeOH as solvent B in 15 min of analysis in total.

As shown in Table 3, the elution gradient and its timepoints are reported.

2.6 Cell culture

Human neuroblastoma SH-SY5Y cells were obtained from ATCC (Manassas, VA) and cultured in a humidified incubator at 37°C with 5% CO_2. The SH-SY5Y cells were grown in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin, L-glutamine (2 mM), non-essential amino acids (1 mM), and sodium pyruvate (1 mM).

RPMI 8226 human multiple myeloma cells (ATCC, Manassas, VA, United States) were cultured in RPMI 1640 medium (Euroclone, Pero, Italy) supplemented with 10% fetal bovine

serum (FBS; GibcoR, Thermo Fisher Scientific, Lisbon, Portugal), 1% glutamine, and 1% penicillin and streptomycin (Euroclone, Pero, Italy). The cells were incubated at 37°C with 5% CO_2 in a humidified incubator. (*R*)-ASME was dissolved in phosphate-buffered saline (PBS) at 1 g/ml concentration and then diluted directly into the culture medium to working concentrations.

2.7 Western blotting

SH-SY5Y cells were treated with 100 nM and 1 μM (*R*)-ASME for 1, 2, and 4 h and with 100 nM and 1 μM (*R*)-germichrysone for 4 h. Then, the cells were homogenized in a specific lysis buffer (Cell Signaling Technology, Denver, CO, United States). Proteins were diluted in 2× sodium dodecyl sulfate (SDS) protein gel loading solution, boiled for 5 min, separated onto 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE)), and processed following standard procedures. In each well, we loaded 40 μg of the total homogenate. The mouse monoclonal antibodies were diluted as follows: the anti-ELAVL4/HuD antibody (Sigma-Aldrich, Darmstadt, Germany SIGMA) at 1:1000; the anti-BDNF antibody (anti-brain-derived neurotrophic factor; Sigma-Aldrich) at 1:500; the anti-p62 antibody (Santa Cruz Biotech, Santa Cruz, California) at 1:1000; the anti-Ub antibody (anti-ubiquitin antibody; Santa Cruz Biotech) at 1:200; and the anti-α-tubulin antibody (Sigma-Aldrich) at 1:1000. The nitrocellulose membrane signals were detected via chemiluminescence (by using WesternBright® ECL HRP substrate, Advansta, San Jose, CA, United States) by means of an Imager Amersham 680 detection system. Alpha-tubulin was used for data normalization. We used folic acid as a positive control, a compound that we previously demonstrated to be able to positively affect the protein expression of both HuD and BDNF (Marchesi et al., 2023). Statistical analysis was performed on the densitometric values obtained with the ImageJ image processing program.

2.8 Proteasome activity assay

2.8.1 Enzymatic assay

The activity of (*R*)-ASME on the 20S proteasome, specifically its chymotrypsin-like catalytic site, was measured via a fluorogenic, non-radioactive and commercially available kit "20S Proteasome Assay Kit for Drug Discovery" (Enzo® Life Sciences, Farmingdale, NY, United States, BML-AK740). Briefly, a solution of 20S proteasome at 10 μg/ml was prepared in Assay Buffer together with a 75 μM solution of the Suc-LLVY-AMC substrate and a 0.5 μM solution of epoxomicin in Assay Buffer. Several dilutions of (*R*)-ASME were chosen to be tested: 3 μM, 30 μM, 300 μM, 1 mM, 3 mM, 5 mM, and 10 mM. A 96-well plate was seeded as follows: blank (no proteasome, 40 μL of Assay Buffer and 10 μL of the fluorogenic substrate), control (no inhibitor, 30 μL of Assay Buffer, 10 μL of 20S proteasome solution, and 10 μL of the fluorogenic substrate), inhibitor (25 μL of Assay Buffer, 10 μL of 20S proteasome solution, 5 μL of epoxomicin, and 10 μL of the fluorogenic substrate), and test samples (25 μL of Assay Buffer, 10 μL of 20S proteasome solution, 5 μL of (*R*)-ASME dilutions, and 10 μL of the fluorogenic substrate). The analysis was performed in triplicate. After seeding, the plate was incubated at 30°C for 10 min to permit the inhibitor/enzyme interaction. After incubation, the substrate was added, starting the cleavage reaction. Excitation was set at 360 nm, and emission was measured at 460 nm with a FLUOstar Omega filter-based multi-mode microplate reader (BMG LABTECH Ltd., Aylesbury, UK). Data were recorded at 1-min intervals over 60 min of analysis and were subsequently analyzed following the manufacturer's instructions: the data were plotted as arbitrary fluorescence unit/min (AFU/min), using a linear regression program. A line fitting considering only the timepoints in which the reaction is linear (from 0 to 10–15 min) was made and its slope calculated. The activity was obtained applying the following equation: 100-[(inhibitor slope/control slope)/x100]. Since (*R*)-ASME is a proteasome activator, the percentage was over 100%.

2.8.2 Cellular assay for proteasome activity

RPMI 8226 cells were seeded at a density of 250×10^3 cells/well in 6-well plates. After 24 h, the cells were treated with (*R*)-ASME (1–1000 mg/ml, i.e. 3 mM–3 M). At 24 h, the cells were collected, and the total protein extract was obtained. The lysis buffer used to obtain total protein extract was HEPES 5 mM pH 7.5, NaCl 150 mM, glycerol 10%, Triton ×100 1%, $MgCl_2$ 1.5 mM, and EGTA 5 mM.

The protein content was quantified with the Bradford method. Forty micrograms of proteins, 10 μL of 10× proteasome buffer (HEPES pH 7.5 250 mM, EDTA pH 8.0 5 mM, NP-40 0.5%, and SDS 0.01%), and 10 μL of the proteasome substrate (N-succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin, 7.6 mg/ml) (Sigma-Aldrich, United States) were loaded in each well of a black 96-well plate. After 2 h at 37°C, fluorescence was quantified in a microplate reader (excitation 380 nm; emission 460 nm) (BMG LABTECH, Germany).

2.9 Statistical analysis

The GraphPad Prism statistical package (version 9, San Diego, CA, United States) was used for statistical analysis. The data were analyzed by analysis of variance (ANOVA) followed, when significant, by an appropriate *post hoc* comparison test, as detailed in the figure legends. Differences were considered statistically significant when p-value ≤0.05. The results are expressed as mean ± S.E.M.

3 Results

3.1 MCLib-2022 virtual screening

The computational approach pursued to identify dual ligands uses the SAFAN platform and docking experiments. SAFAN is a ligand-based method that evaluates the molecular similarity between the investigated molecules and the active compounds collected in the database. Briefly, the following steps were foreseen: molecular fragmentation, quantitative target affinity calculation, based on fragment and compound similarity, and WEKA machine learning approach. The output of the analysis is a list of potential targets, ranked in decreasing order (Supplementary Material). The compounds screened to identify new HuD binders belong to an in-house library (MCLib-2022) (Pagano et al., 2023). This library

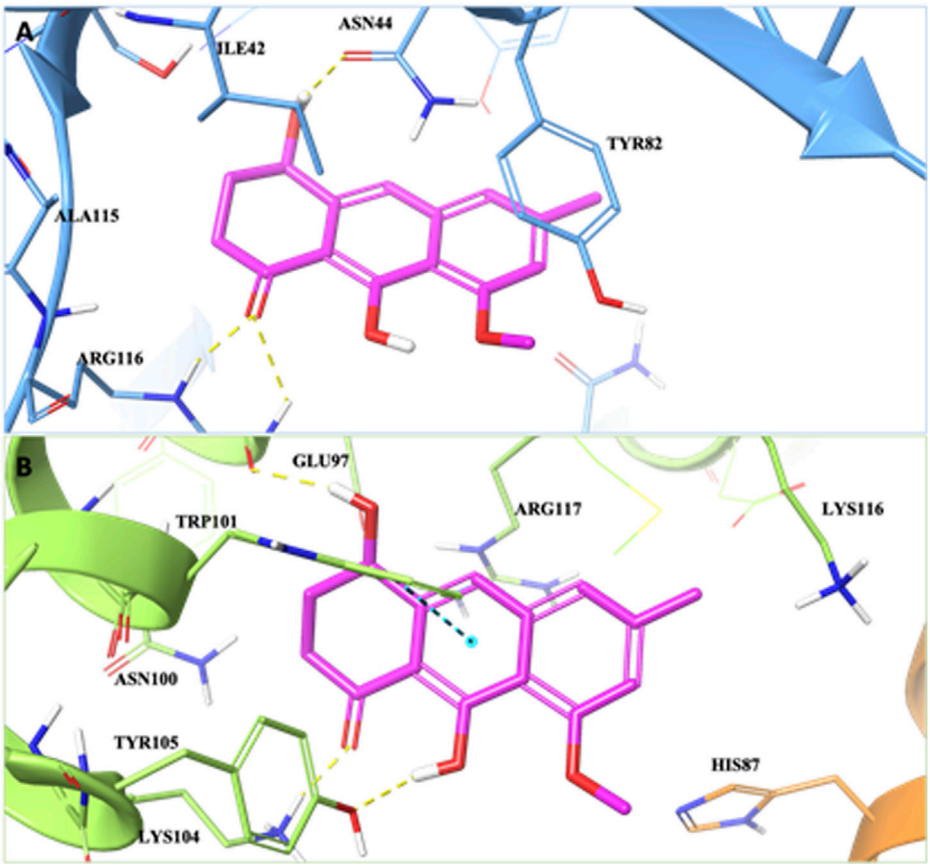

FIGURE 1
3D representation of the (*R*)-ASME complex in the **(A)** HuD binding pocket and **(B)** the α2/α6 intersubunit pocket of the 20S proteasome α-ring. The protein HuD protein is shown as blue, and α2/α6 subunits are shown as orange and green, respectively. The ligand is shown as magenta sticks. The residues involved in crucial contacts with the compounds are reported, respectively, as blue, orange, and green carbon sticks. Hydrogen bonds and π−π stacking interactions are reported, respectively, as yellow and cyan dashed lines.

accounts for ca. 500 non-commercial small molecules synthesized or isolated from natural matrices by the authors' research group. After computational analysis, nine main chemical clusters were identified within the library, and the representative general chemical structure was subjected to virtual screening. More information is available in the paper by Pagano et al. (2023). The SAFAN results highlighted that (*R*)-ASME, belonging to MCLib-2022, is a potential binder of HuD. Before proceeding to extract the selected metabolite to investigate its *in vitro* profile, we further *in silico* analyzed its interaction not only with HuD but also with the proteasome, in order to evaluate its multi-target potential.

3.2 Molecular recognition studies of (*R*)-ASME *versus* HuD and 20S proteasome α-rings

Molecular recognition studies carried out on HuD highlighted that the ligand is well-accommodated in the protein pocket. In detail, (*R*)-ASME establishes three hydrogen bond interactions: one with Asn44 and two with Arg116 (Figure 1A). Moreover, the compound is engaged in several hydrophobic contacts with Ile42, Tyr45, Tyr82, Lys111, Arg123, Ile152, and Thr153.

In order to identify the putative binding mode of (*R*)-ASME *versus* the α-rings, molecular modeling studies were also carried out on the 20S proteasome. Due to the novelty of the target and the lack of information about the ligand-binding pocket on the α-rings, the SiteMap program was used to identify and characterize the putative ligand-binding sites on the 20S proteasome α-rings.

The molecular recognition results showed that the compound is accommodated in the α2/α6 intersubunit pocket, establishing hydrogen bond interactions with Glu97, Lys104, and Tyr105 and a π−π stacking interaction with Trp101 (Figure 1B). Moreover, (*R*)-ASME is engaged in several hydrophobic contacts with His87, Asn100, Tyr105, Lys116, and Arg117.

Thus, molecular recognition studies, carried out on HuD and α-rings of the 20S proteasome, revealed that the ligand is well-accommodated in both the binding sites.

3.3 Extraction and isolation optimization of (*R*)-ASME

To dispose of a suitable amount of (*R*)-ASME with adequate purity for the biological investigation, as a first step, we optimized the already described extraction procedure of *E. persicus* roots. First,

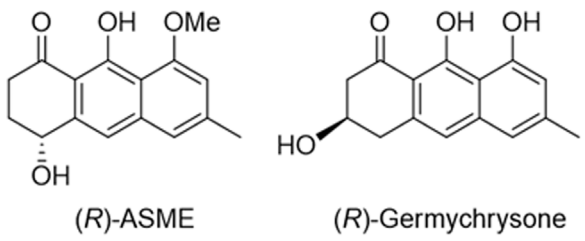

FIGURE 2
Chemical structure of (*R*)-ASME and (*R*)-germichrysone.

an efficient analytical HPLC-UV/PAD method was set up. Considering the good resolution of the main peaks achieved in the previous work, we further optimized the method using the same column (Chromolith SpeedROD RP-18 endcapped column) and varied the gradient applied. As a result, the time of analysis was reduced from 30 to 15 min with no loss of resolution, thus containing the analysis costs and increasing the throughput.

The next step was to optimize the extraction procedure by testing different protocols. Indeed, in our previous work, we developed a MASE protocol that required almost 1 hour, but never investigated the effect of time, temperature, and the heating source on the process. Specifically, the dried and powdered roots of *E. persicus* were subjected to either maceration (Mac) or microwave-assisted solvent extraction (MASE). The latter technique was selected for its several advantages such as higher efficiency and shorter extraction time. Furthermore, different extraction times (two or three cycles of 1 or 12 h for maceration and of 20 or 5 min for MASE) and temperatures (from room temperature to 120°C) were tested. All the raw extracts so obtained were next fractionated via liquid/liquid extraction using water/ethyl acetate. The organic fractions were then dried, evaporated under reduced pressure, and chromatographed to obtain two different pure metabolites. The best results were obtained performing two cycles (2 × 5 min) with solvent renewal at 60°C adopting an MASE approach (MASE_4). Thus, although maceration performed at 60°C is associated with a very high extraction yield, the advantages are not mirrored in the yield of pure metabolites. Indeed, the amount of ASME obtained following methods MASE_4 and Mac_4 is comparable, but microwaves offer a significant improvement in terms of time required (10 min vs. 13 h). This advantage can be explained considering the well-known higher efficiency related to microwave-assisted extraction (Martino et al., 2019; Cavalloro et al., 2021). Two metabolites have been thus isolated and properly characterized by MS and NMR spectroscopy: (*R*)-ASME and enantiomeric germichrysone, a secondary metabolite already identified in species belonging to the genus *Cassia* (Figure 2).

3.4 Assignment of absolute configuration of germichrysone

The absolute configuration (AC) of germichrysone, extracted from *Cassia torosa* Cavanilles, was determined from crystallographic data as (*R*), but in the same work, no optical rotation data were reported (Noguchi et al., 1978). Two years earlier, another work had measured negative optical rotation (-38, dioxane, c 0.52) for germichrysone extracted from the same plant (Takahashi et al., 1976). Conversely, the optical rotation of germichrysone extracted from *E. persicus* measured in tetrahydrofuran gave a positive value (Supplementary Table S3). Considering the opposite rotation of the compound extracted from *E. persicus* with respect to literature data and the low measured value, its AC was herein studied by means of full chiroptical spectroscopy encompassing also vibrational circular dichroism (VCD), electronic circular dichroism (ECD), and optical rotatory dispersion (ORD), together with their prediction via DFT and TD-DFT quantum mechanical calculations (Abbate et al., 2009; Nafie, 2011; Polavarapu, 2016; Mazzeo et al., 2017; Santoro et al., 2019). Moreover, since germichrysone is a fluorescent compound, its circularly polarized luminescence (CPL) activity was also measured.

The ECD spectrum (Figure 3, middle) exhibits a weak positive band at ca. 375 nm, a second weak positive band allied to the UV feature centered at 325 nm, followed by a sequence of two negative bands at 275 nm and 220 nm. TD-DFT calculations satisfactorily are in agreement with experimental spectra in ECD band signs, relative magnitude, and position, thus supporting (*R*)-AC. In Supplementary Figures S1, S2, we report all conformers' calculated structures and corresponding ECD spectra, respectively. Six most stable conformers were found (Supplementary Table S2): three with the OH group in the cyclohexanone ring in equatorial conformation and three in axial conformation, corresponding to the three rotameric states of the aliphatic hydroxyl group. Equatorial and axial conformers contribute differently to averaged ECD in an almost enantiomeric fashion, making the calculation of ECD to be dramatically sensitive to conformer relative population evaluation. For this reason, also ORD and VCD-IR spectra have been considered. OR has been measured at three different wavelengths (Supplementary Table S3): +12 at 589 nm, +18 at 546 nm, and +108 at 436 nm. Quantum mechanics calculations of OR values (assuming (R)-AC) give a good prediction of sign and order of magnitude, thus confirming (R)-AC, as reported in the ECD-UV case.

Complementary to ECD data, it is also interesting to consider the CPL and fluorescence spectra (Figure 3, right). A structured fluorescence band is centered at 480 nm (showing a ca. 100 nm Stokes shift with respect to the low-energy UV band set at 375 nm); the CPL spectrum shows a positive band matching the sign of the low-energy experimental ECD band (Figure 3, middle); this is the most standard behavior observed for organic compounds, in which the ground and excited states retain a similar structure (Longhi et al., 2016). The dissymmetry ratio g_{lum} (defined as Δ/I) is quite low (ca. $4{\cdot}10^{-4}$) but larger than its absorption counterpart g_{abs} (evaluated as Δε/ε for the lowest energy transition CD band), which is ca. $6{\cdot}10^{-5}$. It is also worth noting that the large Stokes shift experienced suggests an excited state intramolecular proton transfer process, which was recently investigated through fluorescence and CPL in natural compounds (Mazzeo et al., 2023).

Finally, to provide further evidence for the assigned AC, VCD and IR spectra were also measured and calculated (Figure 3, left). The main experimental VCD and IR features (IIII) are

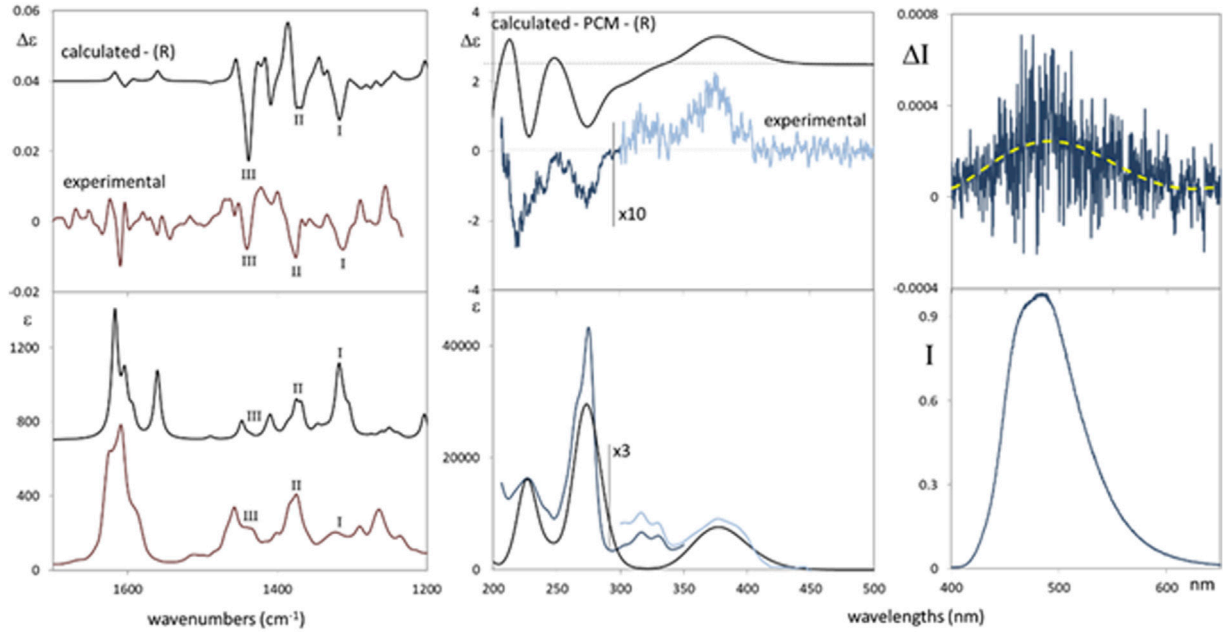

FIGURE 3
Comparison of the experimental and calculated VCD-IR (left panels) and ECD-UV (middle panels) spectra of germichrysone. For better comparison, light blue experimental spectra (500–300 nm range) were multiplied by a factor 10 for ECD (middle, top-right) and by a factor 3 for UV (middle, bottom right). Calculations, on (*R*)-germichrysone, were performed at the TD-DFT/CAM-B3LYP/TZVP level for ECD-UV and at DFT/B3LYP/TZVP for VCD-IR. The calculated ECD-UV spectra are red-shifted by 25 nm. The VCD-IR applied scaling factor is 0.97. Right panels: CPL (top right) and fluorescence (bottom right) spectra of germichrysone. Solvent: tetrahydrofuran. The excitation wavelength is 375 nm. The fluorescence band maximum is normalized to 1.

well-predicted. However, the experimental IR band I, at ca. 1315 cm^{-1}, is overestimated by the calculation, although it is well-predicted in VCD as a negative band. Experimental bands II and III, at ca. 1370 cm^{-1} and 1430 cm^{-1}, respectively, also provided negative features in the experimental VCD spectrum and are satisfactorily predicted by the calculation. Taken together, obtained VCD-IR results support (*R*)-AC for germichrysone, in agreement with ECD and ORD investigations.

3.5 *In vitro* assessment

3.5.1 Ability to bind HuD

The effect of 100 nM and 1 μM (*R*)-ASME on HuD and BDNF protein expression was evaluated at different timeframes, namely, 1, 2, and 4 h. The same investigation was carried out for (*R*)-germichrysone at the same concentrations (1 μM and 100 nM) but only at 4 h. The concentrations and times of exposure were chosen according to our previous published paper (Marchesi et al., 2023) and to our own experience as being optimal for BDNF transcriptional activity, mRNA half-life (t1/2 132 ± 30 min), and protein expression (see also Castrén et al., 1998).

The results obtained show that (*R*)-ASME is able to significantly increase HuD (Figure 4A) and BDNF (Figure 4B) levels at both concentrations following 4-h incubation. Conversely, (*R*)-germichrysone negatively affects the expression of both HuD and BDNF proteins at both tested concentrations after 4-h exposure (Supplementary Figure S4).

3.5.2 Proteasome enzymatic activity

The direct interaction of (*R*)-ASME with the chymotrypsin-like protease active site of the 20S proteasome was evaluated by exploiting the commercially available 20S Proteasome Assay Kit for drug discovery. (*R*)-ASME is able to directly activate the 20S proteasome in a dose-dependent manner. In particular, (*R*)-ASME enhances the activity 6-fold at 300 μM, reaching a plateau at 3 mM with a 26-fold increase (Figure 5).

3.5.3 Proteasome activity in RPMI cells

The effect of (*R*)-ASME on the proteasome was initially tested on RPMI cells. (*R*)-ASME was able to enhance proteasome activity in a dose-dependent manner, doubling the activity at 3 mM and tripling it at 30 mM (Figure 6).

3.5.4 Proteasome activity in SH-SY5Y cells

The effect of 100 nM and 1 μM (*R*)-ASME on p62 expression was evaluated at different timeframes. As shown in Figure 7 (panel A), (*R*)-ASME was able to significantly reduce the level of p62, which is an indicator of protein breakdown via the proteasome, after 4 h at both concentrations. Notably, the decrease of p62 is an index indicating that the degradation phase was successful, while its increase reveals that the degradation has not occurred. The levels of the ubiquitination of folded/unfolded proteins that will undergo degradation and the effect of (*R*)-ASME on this process were also investigated at the same timeframes and concentrations. As shown in Figure 7 (panel B), (*R*)-ASME is able to induce a trend toward a decrease, thus indicating that the degradation phase was successful.

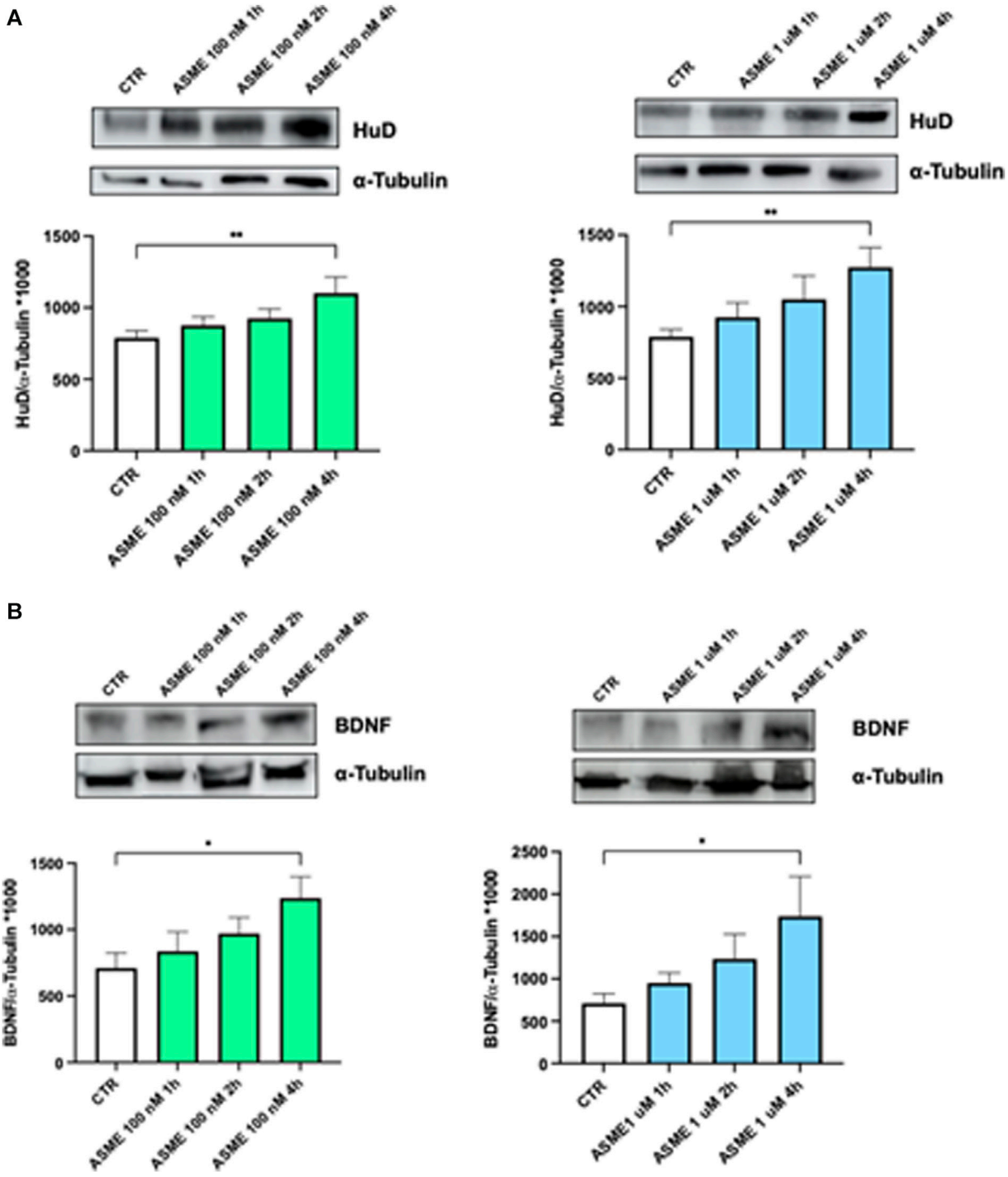

FIGURE 4
HuD and BDNF expression following 1, 2, and 4 h of (*R*)-ASME treatment. **(A, B)** Densitometric analysis of HuD **(A)** and BDNF **(B)** proteins and the respective α-tubulin in the total homogenates of SH-SY5Y cells following exposure to solvent (0.1% of DMSO; CTR) or (*R*)-ASME (ASME) 100 nM (left) and 1 μM (right) for 1 h, 2 h, and 4 h. The results are expressed as mean gray level ratios (mean ± S.E.M.) of HuD/α-tubulin **(A)** and BDNF/α-tubulin **(B)** ×1000. *$p < 0.05$, **$p < 0.01$, Dunnett's multiple comparisons test, n = 6 independent samples.

4 Discussion

Despite the great efforts of the scientific community to discover new effective drugs against neurodegenerative diseases, there is still a lack of effective cures for these pathologies. Therefore, there is an urgent need for molecules acting through novel and not fully explored mechanisms of action, thus enabling a significant breakthrough in this field. Among the recently explored targets, here we focused on the HuD protein and the proteasome system, which may represent valuable candidate targets for the treatment of several relevant neurodegenerative diseases. HuD is still poorly investigated from a medicinal chemistry perspective, and only a

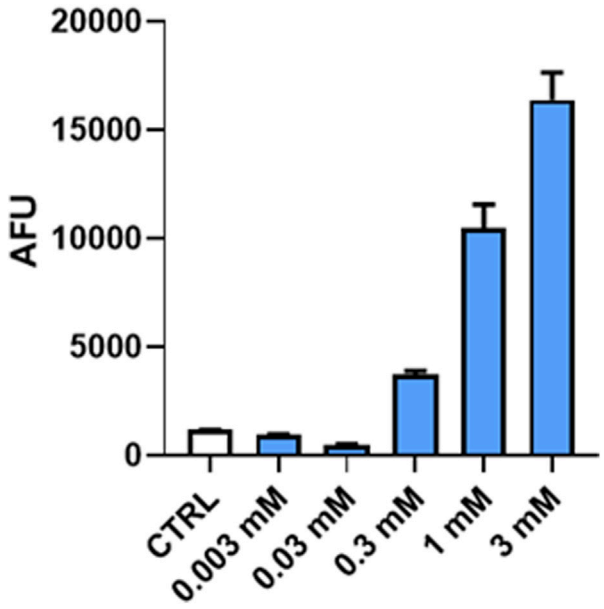

FIGURE 5
Plot of arbitrary fluorescence units (AFUs) against (*R*)-ASME concentration [mM] at a 10-min time point. The results are expressed as AFU mean (mean ± S.E.M.).

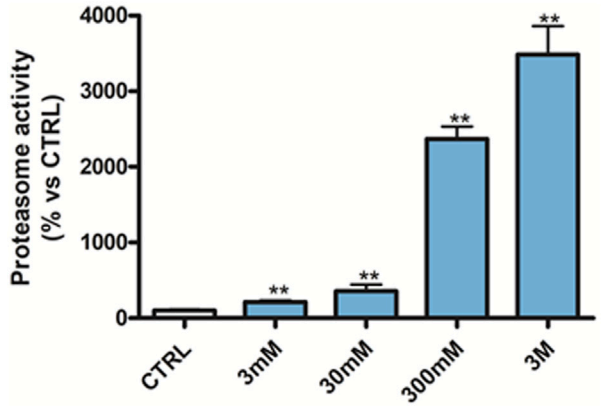

FIGURE 6
Proteasome activity in RPMI 8226 cells following 24 h of 3 mM–3 M (*R*)-ASME treatment. The results are expressed as mean percentage (mean ± S.E.M.) compared to untreated CTRL. $^{**}p < 0.01$, Dunnett's multiple comparisons test, n = 3 independent experiments.

few molecules able to bind this target have been identified by now (Ambrosio et al., 2021; Silvestri et al., 2022; Zhang et al., 2023). Among these, folic acid (a vitamin widely used in the prevention of some neurodegenerative diseases) is worth mentioning as a modulator of HuD activity (Ambrosio et al., 2021; Marchesi et al., 2023).

To find new actives potentially able to interact with HuD, we screened the in-house library MCLib-2022, which groups all the molecules synthesized or isolated from natural matrices by our research group along 30 years of medicinal chemistry research activity and thus covering a wide chemical space. Computational studies showed that (*R*)-aloesaponol-III-8-methyl ether [(*R*)-ASME] has the potential to bind the HuD protein and to interact with the ubiquitin–proteasome system, a potential target useful in the development of agents to counteract neurodegenerative diseases. Briefly, the secondary metabolite (*R*)-ASME has been identified from *Eremurus persicus* Jaub & Spach) Boiss by our group in a drug discovery campaign (Rossi et al., 2017). This plant is well-known by the local people for its beneficial effects on human health, and traditional medicine recognizes different activities depending on the part of the plant considered (Beiranvand and Beiranvand, 2022). Of note, the neuroprotective activity of *E. persicus* extracts has never been documented before, thus representing a new potential application.

Prior to the biological investigation, we performed *in silico* analysis to deeply investigate its interaction with the above-mentioned targets. Docking results confirmed the multi-target potential profile of (*R*)-ASME. In fact, we highlighted that the compound is well-accommodated in both the HuD binding site and in the α2/α6 intersubunit pocket of the 20S proteasome. The direct interaction with the selected target is an important feature of (*R*)-ASME, as non-specific interactions are often associated with several side effects and poor efficacy.

To investigate its potential effect on HuD and the proteasome system and to dispose of a suitable amount with adequate purity for the biological investigation, we optimized the previously described extraction procedure of *E. persicus* roots. The new protocol allowed us to shorten both the extraction and analysis time and to simplify the fractionation protocol. Furthermore, we isolated and identified the structurally related germichrysone together with (*R*)-ASME. We then assigned the (*R*)-AC to germichrysone by means of full chiroptical spectroscopy investigation including VCD, ECD, and ORD, together with their prediction by DFT and TD-DFT quantum mechanical calculations, with all these methods tested as the most reliable ways to assign the AC to compounds in solution (Abbate et al., 2009; Nafie, 2011; Polavarapu, 2016; Mazzeo et al., 2017; Santoro et al., 2019). Moreover, since germichrysone is a fluorescent compound, we also measured and detected its CPL activity. Notably, this is the first time (*R*)-germichrysone has been isolated from the genus *Eremurus*.

The *in vitro* investigation was carried out on both compounds, (*R*)-ASME and (*R*)-germichrysone. First, their effect on the HuD protein levels was evaluated, and then the investigation was extended to BDNF, considering that the BDNF transcript is a HuD target and that its corresponding protein is implicated in several neuronal diseases and involved in the regulation of neuronal development, survival, and function. Consistent with SAFAN-ISP prediction and docking evaluation, (*R*)-ASME exposure leads to a significant increase in the HuD protein amount. Moreover, once bound by (*R*)-ASME, the HuD protein is activated, exerting a positive effect on its downstream target BDNF, and likely on itself, in line with our previously published data (Marchesi et al., 2023). Taken together, these findings strongly suggest that (*R*)-ASME is able to effectively regulate the HuD/BDNF cascade. This outcome is of particular interest, as HuD is still a poorly investigated target, and the number of its already identified binders is limited. Considering this, its identification could represent a step ahead in the study of the structure–activity relationship of HuD binders. Conversely, (*R*)-germichrysone, although structurally related to (*R*)-ASME, is not active.

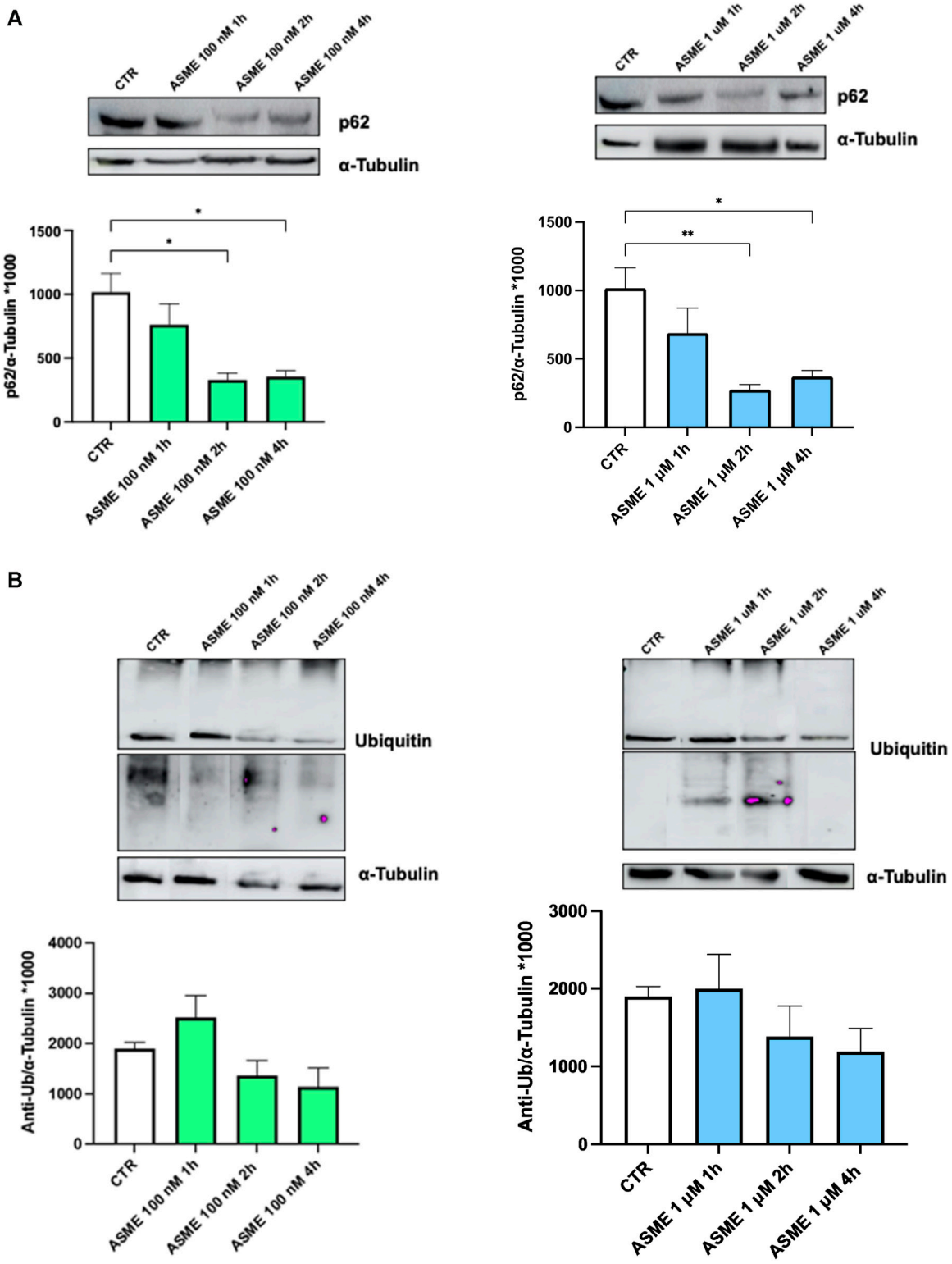

FIGURE 7
P62 and ubiquitin expression following 1, 2, and 4 h of (*R*)-ASME treatment. **(A, B)** Densitometric analysis of p62 **(A)** and ubiquitin (anti-Ub; **(B)** proteins and the respective α-tubulin in the total homogenates of SH-SY5Y cells following exposure to the solvent (0.1% of DMSO; CTR) or 100 nM (left) and 1 μM (right) (*R*)-ASME (ASME) for 1 h, 2 h, and 4 h. The results are expressed as mean gray level ratios (mean ± S.E.M.) of p62/α-tubulin **(A)** and ubiquitin/α-tubulin **(B)** ×1000. *$p < 0.05$, **$p < 0.01$, Dunnett's multiple comparisons test, n = 6 independent samples.

Regarding the effect of (*R*)-ASME on the proteasome system, we demonstrated that this compound directly activates the 20S proteasome. Of note, unlike the 26S proteasome, which degrades ubiquitinylated proteins, the 20S proteasome is able to degrade only disordered proteins, and many of its enhancers have already been identified in George and Tepe (2021). The chemical structure of these molecules may vary, but no enhancers structurally related to (*R*)-ASME have been identified yet. Further results obtained in RPMI (a cell line in which the proteasome activity is highly dysregulated) and in SH-SY5Y cells clearly demonstrate the ability of (*R*)-ASME to operate as an activator of the proteasome complex, as evidenced by the decreasing of both the expression of both p62 (an indicator of degradation) and ubiquitin (a marker of proteins undergoing degradation).

The dual nature of (*R*)-ASME could make this molecule suitable for fighting multifactorial diseases such as neurodegenerative diseases.

5 Conclusion

In the present work, we have identified (*R*)-ASME as a multi-target ligand, potentially useful in counteracting neurodegenerative diseases, characterized by closely related mechanisms of action: the ability to act on the HuD/BDNF cascade and to activate the ubiquitin–proteasome system, two orthogonal mechanisms that may act in an additive/synergistic manner. The results of the molecular recognition studies confirm those obtained by preliminary virtual screening and, more importantly, consistent with those obtained by virtual screening. Overall, these results suggest an important prospective use of (*R*)-ASME in neurodegenerative diseases, particularly those characterized by an altered protein degradation system, such as Alzheimer's disease. Furthermore, the results presented here lay the foundation for the identification of novel hit/lead compounds with a multi-target profile.

These results also pave the way for the development of new precision medicine. Thus, this approach is still poorly applicable in the neurodegenerative field, as it usually focuses on a single-target perspective, whereas a multi-target approach may be required for neurodegenerative diseases with complex molecular pathways.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

Author contributions

VC: data curation, investigation, methodology, and writing–original draft. NM: data curation, investigation, methodology, and writing–original draft. PL: investigation and writing–original draft. DR: investigation and writing–original draft. LC: methodology and writing–original draft. AF: methodology and writing–original draft. KA: methodology and writing–original draft. AM: methodology and writing–original draft. MM: investigation and writing–review and editing. GM: investigation, methodology, writing–original draft, and formal analysis. SA: writing–review and editing. GL: writing–review and editing. FA: investigation, methodology, writing–original draft, and data curation. GC: writing–review and editing and investigation. SA: writing–review and editing. AP: investigation and writing–review and editing. EM: conceptualization, project administration, writing–review and editing, and supervision. SC: conceptualization, project administration, writing–review and editing, and supervision.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors acknowledge SAFAN-ISP (*In Silico* profiling) for the virtual screening.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

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TYPE Review
PUBLISHED 05 February 2024
DOI 10.3389/fphar.2023.1285967

OPEN ACCESS

EDITED BY
Oriana Awwad,
The University of Jordan, Jordan

REVIEWED BY
Akila Prashant,
JSS Academy of Higher Education and Research, India
Yazun Jarrar,
Al-Balqa Applied University, Jordan

*CORRESPONDENCE
Chonlaphat Sukasem,
chonlaphat.suk@mahidol.ac.th

†These authors have contributed equally to this work

RECEIVED 30 August 2023
ACCEPTED 26 December 2023
PUBLISHED 05 February 2024

CITATION
Biswas M, Vanwong N and Sukasem C (2024), Pharmacogenomics and non-genetic factors affecting drug response in autism spectrum disorder in Thai and other populations: current evidence and future implications.
Front. Pharmacol. 14:1285967.
doi: 10.3389/fphar.2023.1285967

Pharmacogenomics and non-genetic factors affecting drug response in autism spectrum disorder in Thai and other populations: current evidence and future implications

Mohitosh Biswas[1,2,3†], Natchaya Vanwong[4,5†] and Chonlaphat Sukasem[2,3,6,7,8*]

[1]Department of Pharmacy, University of Rajshahi, Rajshahi, Bangladesh, [2]Division of Pharmacogenomics and Personalized Medicine, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, [3]Laboratory for Pharmacogenomics, Ramathibodi Hospital, Somdech Phra Debaratana Medical Center SDMC, Bangkok, Thailand, [4]Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand, [5]Cardiovascular Precision Medicine Research Group, Special Task Force of Activating Research (STAR), Chulalongkorn University, Bangkok, Thailand, [6]Pharmacogenomics and Precision Medicine Clinic, Bumrungrad Genomic Medicine Institute (BGMI), Bumrungrad International Hospital, Bangkok, Thailand, [7]Faculty of Pharmaceutical Sciences, Burapha University, Mueang, Thailand, [8]Department of Pharmacology and Therapeutics, MRC Centre for Drug Safety Science, Institute of Systems, Molecular, and Integrative Biology, University of Liverpool, Liverpool, United Kingdom

Autism spectrum disorder (ASD) may affect family and social life profoundly. Although there is no selective pharmacotherapy for ASD, the Food and Drug Administration (FDA) has recommended risperidone/aripiprazole to treat the associated symptoms of ASD, such as agitation/irritability. Strong associations of some pharmacokinetic/pharmacodynamic gene variants, e.g., *CYP2D6* and *DRD2*, with risperidone-induced hyperprolactinemia have been found in children with ASD, but such strong genetic associations have not been found directly for aripiprazole in ASD. In addition to pharmacogenomic (PGx) factors, drug–drug interactions (DDIs) and possibly cumulative effects of DDIs and PGx may affect the safety or effectiveness of risperidone/aripiprazole, which should be assessed in future clinical studies in children with ASD. Reimbursement, knowledge, and education of healthcare professionals are the key obstacles preventing the successful implementation of ASD pharmacogenomics into routine clinical practice. The preparation of national and international PGx-based dosing guidelines for risperidone/aripiprazole based on robust evidence may advance precision medicine for ASD.

KEYWORDS

autism spectrum disorder, risperidone, aripiprazole, pharmacokinetic/pharmacodynamic, genetic polymorphisms, pharmacogenomics, precision medicine

1 Introduction

Autism spectrum disorder (ASD) can be categorized as "syndromic ASD," which is associated with morphological signs or symptoms, e.g., restricted, repetitive, and stereotyped patterns of behavior, etc., or as "non-syndromic ASD," alternatively termed idiopathic ASD, which has no associated signs or symptoms (Genovese and Butler, 2020; Aishworiya et al., 2022). The core clinical features of ASD are difficulties in social communication, restricted or fixated interests, and language delays or speech difficulties (Sauer et al., 2021). ASD may affect family and social life profoundly; therefore, it is important to screen all infants and toddlers to identify early signs of ASD at 9 months, 18 months, and again at 24 or 30 months of age, as recommended by the American Academy of Pediatrics (AAP). Well-established and validated rating or assessment scales should be applied for the diagnosis of ASD, such as the scales of the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2). Along with the consideration of the history and clinical presentation of the child, these scales should be applied by trained specialists for the evaluation of ASD (Genovese and Butler, 2020; Aishworiya et al., 2022; Biswas et al., 2022b).

Another well-known 20-point assessment scale is the Modified Checklist for Autism in Toddlers-Revised (M-CHAT-R), developed by the American Association for Child and Adolescent Psychiatry (AACAP). The AACAP recommends checking the risk of ASD through surveillance using this assessment scale for children at the age of 18 and 24 months or when such assessment becomes necessary (Subramanyam et al., 2019). The child is predicted to be at low risk, medium risk, or high risk of ASD if the assessment total score is 0–2, 3–7, or 8–20, respectively. Early screening of symptomatic biomarkers, including developmental, behavioral, cognitive, and body movement/motor developmental-related markers, as described by Subramanyam et al. (2019), may help detect significant ASD symptoms. A thorough diagnostic evaluation is warranted if early detection of ASD symptoms is confirmed (Subramanyam et al., 2019). Recently, Magellan Health adopted clinical practice guidelines for the assessment and treatment of children with ASD that extensively discuss the epidemiology, diagnosis, comorbidity, assessment, pharmacotherapy, and educational and behavioral interventions (Ghani et al., 2020).

Genetic factors governing the predisposition of ASD are under investigation and continue to be firmly established. Some copy number variations (CNVs) may have been associated with the increased risk of developing ASD (Bernier et al., 2016; Zarrei et al., 2019; Bauleo et al., 2021; Costa et al., 2022). A recent whole-exome sequencing study identified one *de novo* causative variant (c.2951G>A) in the *FGD6* gene (OMIM ID: 613520) in Thai ASD patients (Thongnak et al., 2018).

As reviewed by the WHO in 2012, the estimated prevalence of ASD was ~1% globally, although the prevalence rate has been slightly higher (~1·5%–2%) in recent years, as revealed by Lord et al. (2018), DeVane et al. (2019), Turner (2020), and Biswas et al. (2022b). In most developed countries, the distribution of ASD patients shows similar patterns; however, it is comparatively less prevalent in low- and lower-middle-income countries (Biswas et al., 2022b). The increase in the prevalence rate in Western countries over the past several decades has been partly due to changes in diagnostic methods and the inclusion criteria of ASD. Approximately 1 out of 59 children was diagnosed with ASD in the United States of America, and 205,200 children in Australia were diagnosed with ASD in 2018, which represents a ~25% increase in the prevalence rate than that reported in 2015 (Lord et al., 2018; Hyman et al., 2020; Turner, 2020).

However, studies collecting epidemiological data relevant to ASD from low- and lower-middle-income countries are very limited (Hyman et al., 2020). The prevalence rate of ASD in many of these countries is still unknown (Elsabbagh et al., 2012).

There may be some comorbidities or clinical features associated with ASD, including electroencephalogram (EEG) abnormalities with or without epilepsy, intellectual disability (ID), and abnormal findings on magnetic resonance imaging (MRI). Approximately 10% of children with ASD have microcephaly, 28% have attention-deficit/hyperactivity disorder (ADHD), 20% have anxiety disorders, 13% have insomnia disorders, 11% have depressive disorders, 9% have obsessive–compulsive disorder, 5% have bipolar disorders, and 4% have schizophrenia spectrum disorders, as described in some studies. Head enlargement is also common in children with ASD, along with higher brain volumes, especially in the frontal lobes (Genovese and Butler, 2020; Turner, 2020).

Pharmacogenomics (PGx) aims to optimize the overall safety and effectiveness of many clinically recommended conventional medications, considering the genetic variants of drug-metabolizing enzymes, such as cytochrome P450 (CYP) enzymes, or transporter biomolecules affecting the pharmacokinetic or pharmacodynamic properties of the drugs, as evidenced in various studies (Roden et al., 2006; Somogy, 2008; Whirl-Carrillo et al., 2012; Ahmed et al., 2016; Collins et al., 2016; Zhou et al., 2017; Chidambaran and Sadhasivam, 2018; Biswas et al., 2023). It is now well recognized that a 'one-size-fits-all' approach will not be effective for many clinically important medications for certain groups of patients carrying either *CYP* or transporter genetic variants. Instead, a more personalized treatment approach, called precision medicine, achieved through considerations of PGx, is now clinically feasible and operational in many parts of the world (Collins and Varmus, 2015; Relling, 2015; Relling and Evans, 2015; Weinshilboum and Wang, 2017; Blagec et al., 2018; Biswas et al., 2021a; Biswas, 2021; Gong et al., 2021; Sukasem et al., 2023). This narrative review aims to address the therapeutic guidelines, pharmacokinetic/pharmacodynamic properties of ASD medications, current evidence of PGx for ASD medications, and non-genetic factors affecting the safety or effectiveness of ASD medications.

2 Therapeutic guidelines for ASD

ASD, with its complex biological traits, can be difficult to diagnose, especially at the initial stage. Therefore, clinical treatments aimed at compensating for the symptoms associated with ASD are not straightforward. Currently, there are no robust guidelines to follow for ameliorating the symptoms of ASD. However, psychostimulants, atypical antipsychotics, antidepressants, and alpha-2 adrenergic receptor agonists are commonly used to treat core clinical symptoms or manage the symptoms of comorbid conditions in children and adolescents with ASD, as reported by Sharma et al. (2018). The United States Food and Drug Administration (FDA) has approved two drugs,

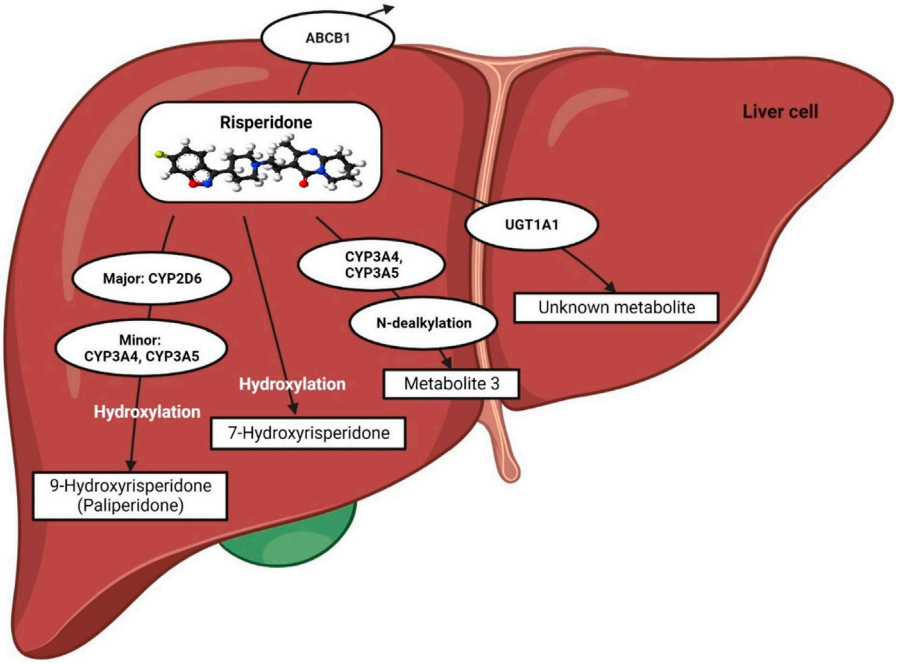

FIGURE 1
Metabolic pathway of risperidone. This figure is adapted from Biswas et al., (Biswas et al., 2022b).

risperidone and aripiprazole, not for the treatment of ASD directly but for alleviating the irritability or agitation symptoms associated with ASD in children and adolescents aged 5–16 years. Risperidone was approved in 2006 by the FDA at a typical dose of 1–3 mg/day. In 2009, aripiprazole was ratified by the FDA at a typical dose of up to 15 mg/day (Riesgo, R., Gottfried, C., & Becker, 2013; Lord et al., 2018; DeVane et al., 2019; Hongkaew et al., 2021b; Biswas et al., 2022b).

3 Clinical problems in ASD treatment

There is no selective therapy for treating the core symptoms of ASD; however, co-occurring health problems commonly reported in ASD, such as attention-deficit/hyperactivity disorder, irritability, agitation, epilepsy, sleep disorders, anxiety, and depression, are usually treated with supportive treatments (Howes et al., 2018; Biswas et al., 2022b). For example, risperidone/aripiprazole is commonly used as a first-line therapy to treat irritability or agitation associated with ASD. However, several adverse effects, e.g., weight gain, increased prolactin level in the blood (hyperprolactinemia), hyperuricemia, leptin disturbance, insulin resistance, and extrapyramidal effects, are commonly reported with the use of risperidone/aripiprazole in children with ASD (Germann et al., 2012; Vanwong et al., 2016c; 2020; Shafiq and Pringsheim, 2018; Aishworiya et al., 2022; Biswas et al., 2022b). Patients might also be at risk of the therapeutic ineffectiveness of the drug. For example, risperidone can be metabolized by the CYP2D6 enzyme, and some phenotypes are potentially being considered CYP2D6 ultra-rapid metabolizers due to the rapid clearance of this drug from the body, as described by Biswas et al. (2022b).

4 Psychopharmacological treatments for ASD

Although the core treatment of ASD is largely dependent on effective behavioral interventions, several potential supportive treatments targeting the underlying neurological disorders of ASD have been the mainstay of ASD management over the last few years (Aishworiya et al., 2022). It has been reported that approximately two-thirds of adolescent ASD patients have been treated with psychotropic medications, especially those diagnosed with neuropsychological problems. Approximately 70% of ASD patients have been found to have several other problems, such as ADHD, irritability, aggression, and mood and anxiety issues, warranting the use of psychotropic medications in these patients (Simonoff et al., 2008; Levy et al., 2010; Feroe et al., 2021; Aishworiya et al., 2022). The following medications are frequently prescribed to ASD children (Aishworiya et al., 2022).

Risperidone: This drug was approved by the FDA in 2006 for children with ASD. It can be prescribed for children older than 5 years of age to reduce the irritability associated with ASD (Riesgo, R., Gottfried, C., & Becker, 2013; Lord et al., 2018; DeVane et al., 2019; Hongkaew et al., 2021b; Biswas et al., 2022b).

Aripiprazole: The FDA approved aripiprazole in 2009 for ASD children who were 6–17 years of age for reducing irritability (Owen et al., 2009; Riesgo, R., Gottfried, C., & Becker, 2013; Lord et al., 2018; DeVane et al., 2019; Hongkaew et al., 2021b; Aishworiya et al., 2022; Biswas et al., 2022b).

Serotonin reuptake inhibitors, anti-anxiety medications, or stimulants: Although the FDA has not recommended the use of selective serotonin reuptake inhibitors (SSRIs, e.g., sertraline, citalopram, fluoxetine, and venlafaxine), tricyclic antidepressants

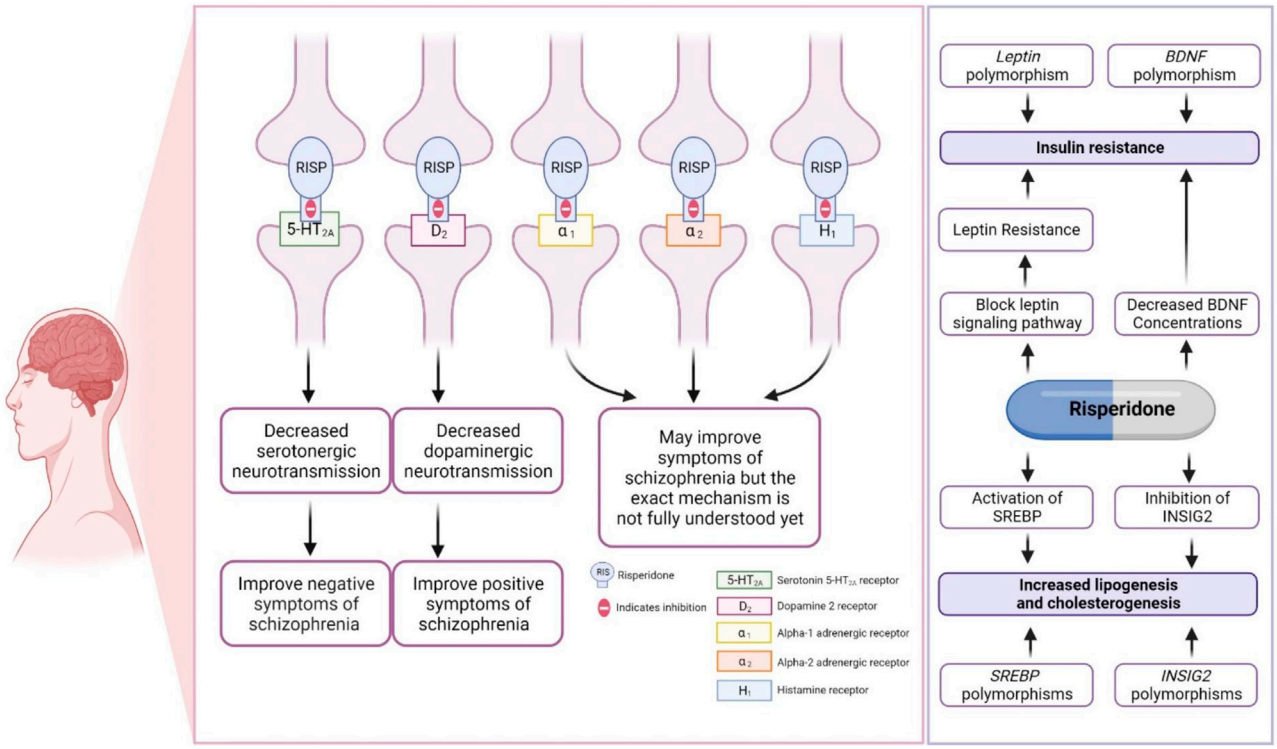

FIGURE 2
Pharmacodynamics of risperidone. This figure is adapted from Biswas et al., (Biswas et al., 2022b).

(TCAs, e.g., amitriptyline and nortriptyline), or stimulants (e.g., amphetamine and methylphenidate) for ASD, some studies have suggested using these medications for certain clinical benefits (Aishworiya et al., 2022). For example, SSRIs may stimulate neurogenesis and produce a neuroprotection effect in ASD children; therefore, some clinicians prefer to use these medications, especially to treat anxiety, mood issues, and irritability associated with ASD (Aishworiya et al., 2022).

Anticonvulsants: Almost one-third of people with ASD have seizures or seizure disorders (Hirota et al., 2014; Besag and Vasey, 2021). Antiepileptic drugs, e.g., carbamazepine and lamotrigine, are commonly prescribed for ASD alongside seizures or seizure disorders. Clinical effectiveness remains controversial (Hirota et al., 2014).

5 Risperidone

5.1 Pharmacokinetics of risperidone

The metabolic pathway of risperidone (a pro-drug) was extensively discussed in our previous review (Biswas et al., 2022b). In short, it is preferentially metabolized by CYP2D6 to a greater extent, whereas CYP3A4/5 enzymes might play a minor role in producing 9-hydroxyrisperidone, known as paliperidone, which is a pharmacologically active moiety (Fang et al., 1999; Berecz et al., 2004; Corena-McLeod, 2015; Puangpetch et al., 2016; Biswas et al., 2022b) (Figure 1).

Risperidone may also act as a substrate and an inhibitor of P-glycoprotein (P-gp), as reported in recent *in vitro* studies (Puangpetch et al., 2016; Soria-Chacartegui et al., 2021). Furthermore, UGT1A1 may also be potentially involved in the metabolic pathway of risperidone since an association between *UGT1A1* genetic polymorphisms and risperidone-induced hyperprolactinemia has been established in a clinical study in Thailand (Hongkaew et al., 2018).

5.2 Pharmacodynamics of risperidone

Risperidone primarily antagonizes the serotonergic (5-HT_{2A}) and dopaminergic (D2) receptors in the brain, although the exact mechanism is not yet fully understood. Risperidone binds ~10–20-fold more preferentially to 5-HT_{2A} receptors than to D2 receptors, and it is considered a potent 5-HT_{2A} receptor antagonist (Leysen et al., 1988; Fenton and Scott, 2005; Canitano and Scandurra, 2008; Kemp et al., 2009; Germann et al., 2012; Corena-McLeod, 2015; Puangpetch et al., 2016; Chopko and Lindsley, 2018; Biswas et al., 2022b). The mechanism by which risperidone reduces associated symptoms of ASD was discussed in detail in our previous review (Biswas et al., 2022b), as shown in Figure 2. Other pharmacodynamic targets, such as brain-derived neurotrophic factor (BDNF) and leptin (LEP), may also be involved in risperidone-induced insulin resistance (Figure 2).

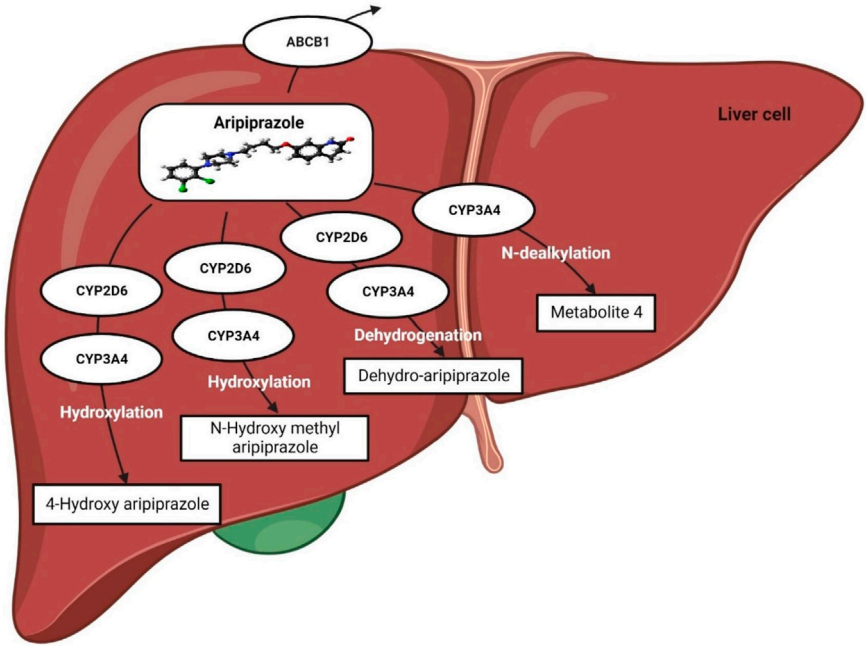

FIGURE 3
Metabolic pathway of aripiprazole.

5.3 Association of *BDNF* and leptin genetic variants with insulin resistance

The *BDNF* gene (OMIM ID: 113505) encoding brain-derived neurotrophic factor (BDNF) and the *LEP* gene (OMIM ID: 164160) encoding leptin (LEP) were found to have a significant association with insulin resistance in ASD patients taking risperidone, suggesting a genetic biomarker for predicting insulin resistance in ASD patients. This association must be replicated in future studies (Sukasem et al., 2018). BDNF has been reported to influence glucose–insulin homeostasis (Tsuchida et al., 2001). Previous studies have reported that decreased BDNF concentrations are found in type 2 diabetes patients (Nakagawa et al., 2000; Krabbe et al., 2007). Interestingly, administration of risperidone has been associated with decreased BDNF levels in the rat brain (Angelucci et al., 2000). The reduction in brain BDNF after being treated with risperidone, along with *BDNF* gene polymorphisms, might be a part of the mechanism causing risperidone-induced type 2 diabetes in people with autism spectrum disorder.

The LEP hormone is a regulator of glucose homeostasis and insulin resistance (Park and Ahima, 2015). Risperidone could reduce both leptin-induced signal transducer and activator of transcription 3 (STAT3) phosphorylation and insulin-mediated protein kinase B activation, which could result in LEP and insulin resistance (Piao et al., 2014). Genetic polymorphisms in *LEP* may affect the safety of risperidone. Although not widely assessed clinically, one study established an association between a SNP of *LEP* (rs7799039) and an increased risk of weight gain in ASD patients taking risperidone (Dos Santos-Júnior et al., 2016; Biswas et al., 2022b). Since the associations have not yet been assessed and replicated in other studies, further studies are needed to confirm the associations of *BDNF* or *LEP* genetic variants with insulin resistance/weight gain in ASD patients treated with risperidone.

5.4 Association of *INSIG2* and *SREBF2* genetic variants with dyslipidemia

Sterol regulatory element binding transcription factor 2 (SREBF2) gene (OMIM ID: 600481) and *insulin-induced gene2* (*INSIG2*) (OMIM ID: 608660) polymorphisms were found to be associated with dyslipidemia in patients treated with risperidone (Vanwong et al., 2021). The *SREBF2* and *INSIG2* genes are involved in the regulation of lipid biosynthesis (DeBose-Boyd and Ye, 2018). Risperidone stimulates both lipogenesis and cholesterogenesis through INSIG2 inhibition and the activation of SREBP2 expression (Cai et al., 2015). *SREBF2* and *INSIG2* might be candidate genes for dyslipidemia in people with autism spectrum disorder treated with risperidone. However, it is necessary to replicate such associations in future studies.

5.5 Clinical outcomes: response and adverse drug reactions of risperidone

The clinical responses and adverse effects of risperidone largely depend on the functional activity of the CYP2D6 enzyme, since risperidone is mainly metabolized by CYP2D6. Some of the less-serious adverse effects of risperidone that are commonly reported are insomnia, anxiety, decreased libido, sedation, dystonia, blurred vision, tachycardia, hypotension/hypertension, and musculoskeletal pain (Soria-

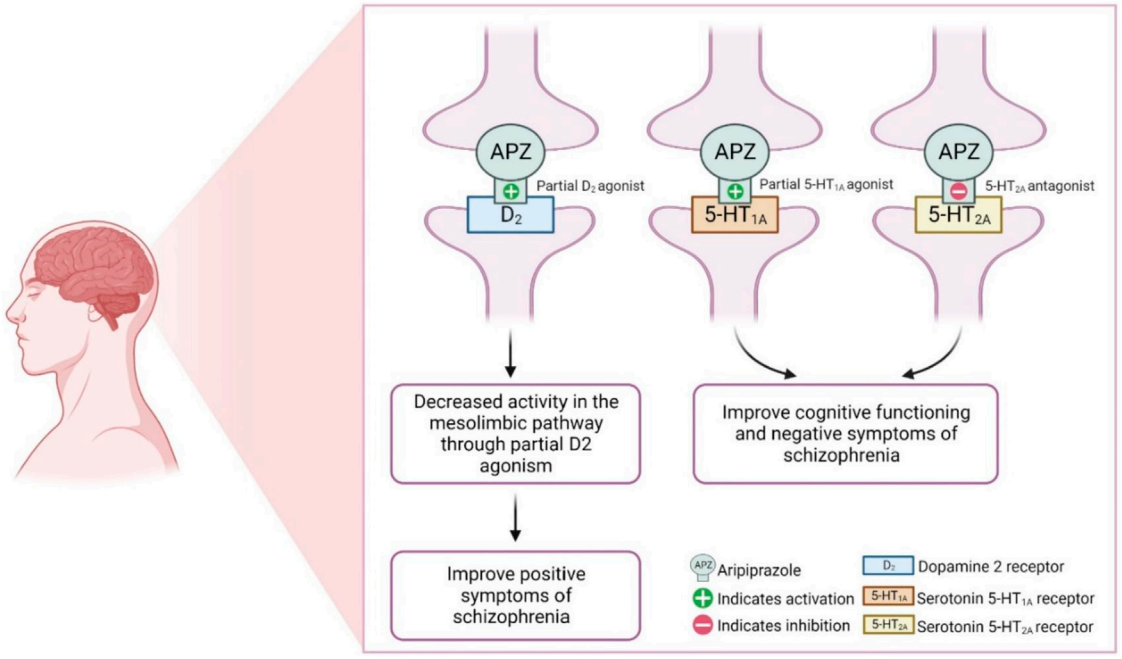

FIGURE 4
Pharmacodynamics of aripiprazole.

Chacartegui et al., 2021; Biswas et al., 2022b). However, the more serious adverse effects of risperidone are weight gain, insulin resistance, hyperprolactinemia, and extrapyramidal effects. These adverse effects may be governed by genetic variants modifying the pharmacokinetic or pharmacodynamic properties of risperidone (Biswas et al., 2022b).

6 Aripiprazole

Aripiprazole, like risperidone, is an atypical antipsychotic mainly used to treat schizophrenia and bipolar disorder; however, it can be used for the management of major depressive disorder and irritability associated with ASD (Dean and Kane, 2012).

6.1 Pharmacokinetics of aripiprazole

Aripiprazole is extensively metabolized in the liver, predominantly by the CYP2D6 and CYP3A4 metabolic enzymes, and converted to dehydroaripiprazole (major metabolite) (Figure 3). The pharmacological activity of aripiprazole is primarily mediated through the parent drug; however, dehydroaripiprazole plays a very minor role in its activity. Aripiprazole takes ~75 h to be eliminated from the body in a normal individual; however, for individuals with poor CYP2D6 activity, i.e., poor metabolizers, it takes ~146 h to be eliminated (Dean and Kane, 2012). It has been reported that in poor metabolizers, the mean aripiprazole exposure may be increased 1.5-fold compared to normal metabolizers (Dean and Kane, 2012; Jukic et al., 2019).

6.2 Pharmacodynamics of aripiprazole

Unlike risperidone, aripiprazole acts as a partial agonist of the dopamine receptor (D2) and has a high affinity for binding like dopamine. However, due to the low intrinsic activity of aripiprazole, it causes very low activation of the D2 receptor compared with dopamine, which favors its use against psychiatric problems. Aripiprazole may reduce the activity of dopamine neurons profoundly in the brain's mesolimbic system due to its high affinity for the D2 receptor and its partial agonist activity. Since overactivity of dopamine causes psychosis and other psychiatric problems, the reduction of dopamine in this region is clinically beneficial in these patients (Potkin et al., 2003; Swainston Harrison and Perry, 2004; Dean and Kane, 2012). In addition, aripiprazole exhibits a strong binding affinity for both 5-HT1A and 5-HT2A receptors. When it binds to the 5-HT1A receptor, aripiprazole acts as a partial agonist, whereas it functions as an antagonist at the 5-HT2A receptor. This mechanism of action could potentially explain the anxiolytic and anti-depressive effects of aripiprazole, as well as its ability to improve cognitive functioning and negative symptoms (Hoyer et al., 2002; Gründer et al., 2006; Dean and Kane, 2012; Muneer, 2016), Figure 4.

6.3 Clinical outcomes: response and adverse drug reactions of aripiprazole

Due to the 5-HT_{2A} antagonistic and D2 agonist activity of aripiprazole, it is primarily indicated in schizophrenia and is also used to treat irritability associated with ASD. Some of the common adverse effects of aripiprazole are suicidal tendencies, neuroleptic

TABLE 1 Clinical annotations and gene polymorphisms of drugs potentially used in ASD.

Level	Variant	Gene	Drug	Phenotype category	Phenotype
Level 1A	*CYP2D6*1, CYP2D6*1xN, CYP2D6*3, CYP2D6*4, CYP2D6*5, CYP2D6*6, CYP2D6*10, CYP2D6*14*	*CYP2D6*	Risperidone	Metabolism/PK	Psychotic disorders and schizophrenia
Level 1A	*CYP2D6*1, CYP2D6*4, CYP2D6*5, CYP2D6*6, CYP2D6*10, CYP2D6*41*	*CYP2D6*	Aripiprazole	Metabolism/PK	Psychotic disorders, schizoaffective disorder, and schizophrenia
Level 1A	*CYP2D6*1, CYP2D6*1xN, CYP2D6*2, CYP2D6*2xN, CYP2D6*3, CYP2D6*4, CYP2D6*5, CYP2D6*, CYP2D6*10, CYP2D6*14, CYP2D6*41*	*CYP2D6*	Paroxetine	Metabolism/PK	-
Level 1A	*CYP2D6*1, CYP2D6*4, CYP2D6*5, CYP2D6*6, CYP2D6*10, CYP2D6*14*	*CYP2D6*	Fluvoxamine	Metabolism/PK	Depressive disorder
Level 3	rs35599367	*CYP3A4*	Risperidone	Metabolism/PK	Bipolar disorder, depression, and substance-related disorders
Level 3	rs887829, rs1976391, rs10929302	*UGT1A1*	Risperidone	Toxicity	Autism spectrum disorder
Level 3	rs1045642	*ABCB1*	Risperidone	Toxicity	Schizophrenia
Level 3	rs1128503	*ABCB1*	Risperidone	Efficacy	Autistic disorder
Level 3	*CYP2D6*1, CYP2D6*4*	*CYP2D6*	Citalopram	Dosage	-
Level 3	rs1065852	*CYP2D6*	Escitalopram	Efficacy	Depressive disorder (major)
Level 3					
Level 3	*CYP2D6*1, CYP2D6*4*	*CYP2D6*	Sertraline	Dosage	Depression
Level 3	rs2032582	*ABCB1*	Fluoxetine	Efficacy	Depressive disorder

PK, pharmacokinetics.

malignant syndrome, hyperglycemia, orthostatic hypotension, leukopenia, neutropenia, agranulocytosis, seizures/convulsions, sedation, and extrapyramidal disorders. (Dean and Kane, 2012).

7 Pharmacogenomics in ASD

7.1 Pharmacogenomics of risperidone

7.1.1 Association of *CYP2D6* genetic variants with risperidone-induced hyperprolactinemia

Since CYP2D6 is the major CYP enzyme involved in risperidone metabolism, the safety or efficacy of risperidone may be affected by *CYP2D6* (OMIM ID: 124030) genetic variants encoding the CYP2D6 enzyme (Puangpetch et al., 2016; de Leon, 2020; Lu et al., 2021; Soria-Chacartegui et al., 2021; Biswas et al., 2022b). Patients who are considered to be CYP2D6 poor metabolizers (PMs) or CYP2D6 intermediate metabolizers (IMs) carrying defective *CYP2D6* alleles might develop higher plasma concentrations of the risperidone/9-hydroxyrisperidone ratio compared to patients considered normal CYP2D6 metabolizers (NMs) or ultra-rapid metabolizers (UMs) (Puangpetch et al., 2016; Soria-Chacartegui et al., 2021). As a consequence, ASD children who are CYP2D6 PMs or IMs might be at higher risk of developing hyperprolactinemia when treated with risperidone (Puangpetch et al., 2016; de Leon, 2020; Soria-Chacartegui et al., 2021; Biswas et al., 2022b). In contrast, patients considered CYP2D6 UMs might be at risk of therapeutic ineffectiveness/failure of risperidone therapy due to a possible reduction of risperidone/9-hydroxyrisperidone plasma concentrations in these phenotypes (Soria-Chacartegui et al., 2021; Biswas et al., 2022b).

7.1.2 Association of *UGT1A1* genetic variants with risperidone-induced hyperprolactinemia

Although the exact metabolic role of UGT1A1 in risperidone metabolism has not yet been elucidated, a recent study found a significant association of hyperprolactinemia with *UGT1A1* (OMIM ID: 191740) genetic polymorphisms in 84 Thai patients with ASD (Hongkaew et al., 2018). Therefore, it is suggested to replicate these findings in other clinical studies of ethnically diverse ASD patients.

7.1.3 Association of *DRD2* genetic variants with risperidone-induced hyperprolactinemia

Since dopamine–D_2-receptor (DRD2) is a pharmacodynamic target of risperidone, the *DRD2* (OMIM ID: 126450) gene encoding this receptor may be associated with risperidone-induced safety or efficacy issues for the patients taking this drug. Lately, a significant association between *DRD2* genetic polymorphisms and hyperprolactinemia has been established in children with ASD (Sukasem et al., 2016; Hongkaew et al., 2021a; Soria-Chacartegui et al., 2021).

7.1.4 Association of *LEP* genetic variants with risperidone-induced weight gain

Genetic polymorphisms in *LEP* may affect the safety of risperidone. It has been found that ASD patients taking risperidone and harboring the rs7799039 SNP of *LEP* (GG genotype) have an increased risk of

TABLE 2 Clinically significant DDIs of ASD therapies, as retrieved from the Medscape Drug Interaction Checker (https://reference.medscape.com/drug-interactionchecker).

Main drug	Interacting drug	DDIs and clinical effects	Recommendation
Risperidone	Sertraline	Sertraline may increase the level or effect of risperidone by affecting drug metabolism through the CYP2D6 pathway	Use with caution/monitor
Aripiprazole		Both risperidone and sertraline increase the QTc interval	Use with caution/monitor
Risperidone	Citalopram	Citalopram and risperidone both increase the QTc interval	Avoid or use an alternate drug
		Citalopram will increase the level or effect of risperidone by affecting hepatic enzyme CYP2D6 metabolism	Use with caution/monitor
Risperidone	Escitalopram	Escitalopram increases the toxicity of risperidone by changing the QTc interval	Use with caution/monitor
Aripiprazole			
Risperidone	Fluoxetine	Fluoxetine will increase the level or effect of risperidone by affecting hepatic enzyme CYP2D6 metabolism	Avoid or use an alternative drug
Aripiprazole	Paroxetine	Fluoxetine and risperidone both increase the QTc interval	Use with caution/monitor
Risperidone	Fluvoxamine	Fluvoxamine and risperidone both increase the QTc interval	Use with caution/monitor
Aripiprazole	Citalopram	Aripiprazole and citalopram both increase the QTc interval	Avoid or use an alternative drug

DDIs, drug–drug interactions; ASD, autism spectrum disorder.

weight gain compared to AA/AG genotypes (Dos Santos-Júnior et al., 2016; Biswas et al., 2022b).

Some other genetic variants, e.g., *ABCB1*, *HTR2C* (OMIM ID: 312861), *CYP3A4/5* (OMIM ID: 124010/605325), and *CNR1* (OMIM ID: 114610), may also affect the safety or effectiveness of risperidone, as reviewed by our group (Biswas et al., 2022b). These pharmacogenomic associations should be replicated in ASD cohorts in future investigations.

7.1.5 Pharmacogenomics of aripiprazole

Since aripiprazole is preferentially metabolized by CYP2D6, the safety or effectiveness of this drug might be affected by *CYP2D6* genetic variability. Recent studies have found an association between *CYP2D6* genetic polymorphisms and hyperprolactinemia, especially in female pediatric populations who are poor *CYP2D6* metabolizers (Grădinaru et al., 2019; Koller et al., 2020). Hyperprolactinemia may significantly affect the growth and development of pediatric populations (Grădinaru et al., 2019), and therefore, these patients need additional monitoring, especially when diagnosed with ASD. Recent case reports found an association between *CYP2D6* activity and atrial fibrillation or abnormal heart electrophysiology (D'Urso et al., 2018; Mazer-Amirshahi et al., 2019), suggesting that *CYP2D6* genetic variants affecting this enzyme activity should be considered in future studies.

7.1.6 Pharmacogenomics of carbamazepine

Since epilepsy appears to be prevalent in ASD patients, antiepileptic drugs, e.g., carbamazepine, might be commonly prescribed to children with ASD. The pharmacogenomic response of carbamazepine in ASD patients has not been assessed yet; however, an association of *HLA-B* (OMIM ID: 142830) pharmacogenomics with carbamazepine-induced SJS/TEN has already been well established (Kloypan et al., 2021; Biswas et al., 2022a) and needs further consideration in ASD patients.

7.1.7 Pharmacogenomics of SSRIs and methylphenidate

Although there is strong evidence for the pharmacogenomic effects of SSRIs, e.g., sertraline, citalopram, and escitalopram, due to *CYP2C19* (OMIM ID: 124020) and *CYP2D6* genetic variability (Hicks et al., 2015), these effects have not been quantified in children with ASD. Future studies should consider these genetic variants in ASD. A recent meta-analysis found statistically significant associations between *ADRA2A* (OMIM ID: 104210), *COMT* (OMIM ID: 116790), and *SLC6A2* (OMIM ID: 163970) genetic variants and the effectiveness of methylphenidate; however, these associations were not pooled from ASD patients (Myer et al., 2018). These genetic variants should be assessed in ASD patients taking methylphenidate.

Clinical annotations of drugs potentially used in ASD with a PharmGKB evidence level are shown in Table 1.

8 Pharmacogenomics interventions in ASD

A very recent PGx study investigated the genetic variants of *CYP1A2* (OMIM ID: 124060), *CYP2C19*, *CYP2D6*, and *SLC6A4* (OMIM ID: 182138) in 42 ASD children who were resistant to pharmacological treatment. The findings of this study revealed that 93% of the ASD children showed improved clinical manifestations after receiving the PGx interventions. Furthermore, 55% of the children in the PGx intervention group achieved stability of clinical symptoms, reducing potential hospital stays and leading to fewer frequent visits to

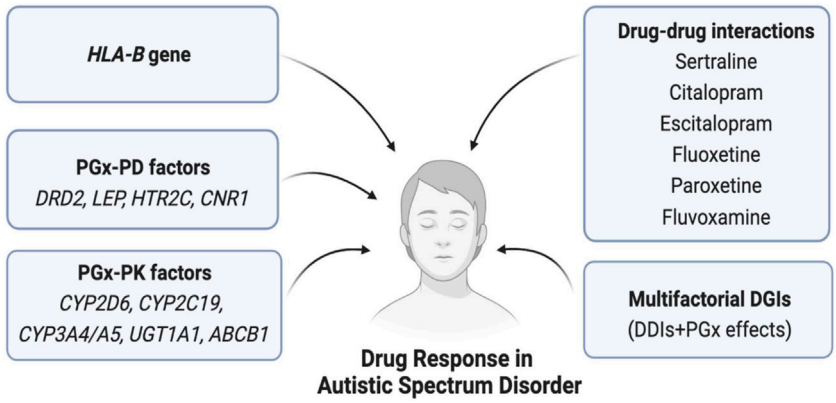

FIGURE 5
Pharmacogenomics and non-genetic factors affecting drug response in ASD. Here, PGx = Pharmacogenomics, PK = Pharmacokinetics, PD = Pharmacodynamics, HLA = Human leukocyte antigen, DGIs = Drug-gene interactions, DDIs = Drug-drug interactions.

their clinicians. This study suggested that PGx interventions have significant potential to improve the clinical manifestations in severe comorbid ASD children who are resistant to the usual drug treatments (Arranz et al., 2022).

8.1 Therapeutic recommendations based on pharmacogenomics testing: updated guidelines

Due to strong genetic associations, pharmacogenomics (PGx)-based dosing guidelines of risperidone clinically indicated for any patients with *CYP2D6* genetic variability have been released by the Dutch Pharmacogenetics Working Group (DPWG) (Beunk et al., 2023). For patients considered *CYP2D6* PMs, the DPWG recommends a dose reduction of risperidone. In contrast, for patients considered *CYP2D6* UMs, the DPWG recommends an alternative antipsychotic drug not primarily metabolized by CYP2D6 or suggests maximizing the dose to achieve the optimum effects (Soria-Chacartegui et al., 2021; Biswas et al., 2022b).

The FDA-approved drug label recommends reducing aripiprazole to 50% of the usual dose for poor *CYP2D6* metabolizers. The DPWG recommends a reduced dose (no more than 10 mg/day or 300 mg/month) for poor *CYP2D6* metabolizers. However, there is no recommendation for intermediate or ultra-rapid metabolizers taking aripiprazole (Dean and Kane, 2012).

9 Non-genetic factors

9.1 Drug–drug interactions

The safety or effectiveness of risperidone in ASD patients may be impacted by clinically significant DDIs, since comorbidities in these patients are likely to be highly prevalent. Risperidone is frequently co-prescribed with antidepressants, anti-epileptics, or other antipsychotics, potentially causing clinically significant DDIs (Puangpetch et al., 2016; Biswas et al., 2022b). Strong CYP2D6 inhibitors (e.g., bupropion) or moderate CYP2D6 inhibitors (e.g., sertraline) may increase the serum concentration of risperidone due to DDIs and potentially cause high blood risperidone-induced adverse effects (Lisbeth et al., 2016). In contrast, CYP2D6 inducer drugs (e.g., rifampin and carbamazepine) may significantly reduce the serum concentration of risperidone if taken concurrently and may cause therapeutic ineffectiveness/failure (Besag and Berry, 2006; Kim et al., 2008; Biswas et al., 2022b). In addition, other mediators affecting the pharmacokinetic properties of risperidone need to be taken into account to avoid potential clinically significant DDIs. For example, the safety or effectiveness of risperidone may be affected if substrate, inhibitor, and inducer drugs of CYP3A4/5 or P-gp are co-prescribed (Kim et al., 2008; Soria-Chacartegui et al., 2021; Biswas et al., 2022b). For aripiprazole, concomitant use of strong CYP2D6/CYP3A4 inhibitors may cause clinically meaningful DDIs, and the prescriber may need to reduce the usual dose of aripiprazole

TABLE 3 Controversy regarding the CYP2D6 activity score range and predicted phenotype for the risperidone metabolism rate between "the consortium (CPIC + DPWG)" and "the PPM-pharmacogenomics of autism spectrum disorders of Thailand Project."

CYP2D6 predicted phenotype based on the combined score	*CYP2D6* activity score range (CPIC + DPWG)	*CYP2D6* activity score range for risperidone in Thai: ASD
Ultra-rapid metabolizer (UM)	>2.25	>2.0
Normal metabolizer (NM)	1.25, 1.5, 2.0, 2.25	**1.0**, 1.25, 1.5, 2.0
Intermediate metabolizer (IM)	0.25, 0.5, 0.75, **1.0**	0.25, 0.5, 0.75
Poor metabolizer (PM)	0	0

CPIC, clinical pharmacogenetics implementation consortium; SSRIs, selective serotonin reuptake inhibitors; PPM, pharmacogenomics and precision medicine, Ramathibodi Hospital, Mahidol University.

(Dean and Kane, 2012). It is also likely to cause cumulative effects due to the combined DDI and *CYP2D6* genetic effects of risperidone, which may further augment the net clinical effects. Although this multifactorial phenomenon called multifactorial drug–gene interactions (DGIs) is clinically feasible and has been evidenced in cardiovascular drugs, e.g., clopidogrel (Biswas et al., 2021b), such combined effects have not yet been quantified in risperidone therapy. To optimize risperidone therapy, it is, therefore, suggested to consider the risk of both DDIs and pharmacogenomics effects of risperidone in future clinical studies. Some of the clinically significant DDIs of ASD therapies are shown in Table 2. Furthermore, the pharmacogenomic and non-genetic factors affecting drug responses in ASD patients are shown in Figure 5.

10 Pharmacogenomics of ASD in Thailand: research and clinical implementation

The prevalence of ASD in Thai children is increasing significantly each year and is potentially increasing the family and social burden (Khaiman et al., 2015; Biswas et al., 2022b). PGx research has progressed considerably in some Asian countries, including Thailand, since many clinically important medications are in routine clinical use in Thailand, where PGx interventions are taken into account (Kloypan et al., 2021; Sukasem et al., 2021; 2023). A large number of clinical studies have already assessed the PGx interference of risperidone in Thai ASD children (Medhasi et al., 2016b; Vanwong et al., 2016b; 2020; Sukasem et al., 2016; 2018; Nuntamool et al., 2017; Srisawasdi et al., 2017; Hongkaew et al., 2018; 2021a; Biswas et al., 2022b). In a prior study, we discovered a significant correlation between the plasma concentration of risperidone and the *CYP2D6* activity score (Vanwong et al., 2016a). These results emphasized the importance of accurately determining a patient's *CYP2D6* genotype-predicted phenotype in clinical settings for the personalized customization of drug therapy (Vanwong et al., 2016a). In addition to examining *CYP2D6* gene polymorphisms, a previous study aimed at exploring genetic variations in drug-metabolizing enzyme and transporter (DMET) genes associated with steady-state plasma concentrations of risperidone among Thai ASD patients found that *ABCB1* (OMIM ID:171050), *ADH7* (OMIM ID: 600086), *SLCO1B1* (OMIM ID: 604843), *SLCO1B3* (OMIM ID: 605495), *SLC7A5* (OMIM ID: 600182), and *UGT2B4* (OMIM ID: 600067) gene polymorphisms were also linked to the plasma concentrations of risperidone. This pharmacogenomic research identified novel genetic variations modulating DMET function that can aid in monitoring risperidone therapy (Medhasi et al., 2016a). In addition, our prior study employed a microarray platform to perform a genetic association analysis of DMET markers with the risperidone-induced prolactin response, evaluated through the hyperprolactinemia and prolactin levels in Thai ASD patients (Hongkaew et al., 2018).

We identified a potential link between *UGT1A1* variants and the prolactin response, which could serve as a foundation for future pharmacogenomic investigations in diverse populations (Hongkaew et al., 2018). In addition to *UGT1A1*, the occurrence of *DRD2 Taq1A* polymorphisms and *DRD2* diplotypes may have a significant effect on the emergence of hyperprolactinemia associated with risperidone use in children and adolescents with a diagnosis of autism spectrum disorder (Sukasem et al., 2016; Hongkaew et al., 2021a). Moreover, the *DRD2* Taq1A polymorphism is linked with a non-stable response to risperidone treatment in patients. This research endorsed the implementation of pharmacogenomics testing to tailor risperidone therapy for individual autistic children and adolescents (Nuntamool et al., 2017). Regarding metabolic adverse effects, a previous study found that gene polymorphisms in leptin and *BDNF* were linked to insulin resistance in Thai children and adolescents with ASD. This implied that leptin and *BDNF* polymorphisms may serve as genetic markers for predicting insulin resistance before commencing treatment in autism spectrum disorder patients receiving risperidone (Sukasem et al., 2018). The overall findings of these studies suggest that PGx screening of some PK/PD genes may be very useful clinically in routine practice to optimize the safety or effectiveness of risperidone in Thai ASD children. Stakeholders and policymakers in Thailand should now focus on the preparation of national PGx guidelines based on the robust evidence from these studies, especially regarding risperidone for Thai ASD children as part of precision medicine (Biswas et al., 2022b).

11 Challenges in pharmacogenomic implementation

11.1 *CYP2D6* discrepancy

The *CYP2D6* allele activity score (AS) varies greatly, and this discrepancy may affect the designation of predicted phenotypes, as discussed extensively in our previous review (Biswas et al., 2022b).The AS of different *CYP2D6* alleles is shown in Supplementary Table S1. The assignment of predicted phenotypes based on the AS of *CYP2D6* has been discussed previously (Biswas et al., 2022b), and the predicted phenotypes based on the combined *CYP2D6* allele AS are shown in Supplementary Table S2.

Novel alleles (i.e., *CYP2D6*142, *143,* and **144*) and a novel sub-allele (*CYP2D6*10.005*) were discovered in the Thai population and have already been recognized by the PharmVar (Hongkaew et al., 2021c), but the ASs of these novel alleles have not yet been assigned. Since the AS may vary, which may affect the assignment of predicted phenotypes accordingly, it is suggested to assess the function of *CYP2D6* genetic variants by measuring the protein expression level for further validation of the predicted phenotypes (Biswas et al., 2022b).

11.2 Polygenic risk score

When multiple genetic variants are involved in determining the clinical response of a drug, the polygenic risk score (PRS) may be a good predictor to assess the safety or effectiveness of that particular drug rather than just considering the effects of each genetic variant separately. The polygenic pharmacogenomics response model might be an integral part of precision medicine development, especially when more than one potential genetic variant will tailor the safety or effectiveness of medications (Lewis et al., 2019; Biswas, 2021; Ikeda et al., 2021). Since multiple PK/PD genetic variants may modify the

clinical response of risperidone/aripiprazole, the PRS approach would be suitable for these drugs and should be considered in future clinical studies.

12 Future perspectives and opportunities

12.1 Pharmacogenomics guidelines

The DPWG has already published PGx-based dosing guidelines for atypical antipsychotics, i.e., risperidone and aripiprazole, not just for ASD, but for all other clinical conditions where these drugs are clinically indicated. However, other international PGx working groups, such as CPIC and CPNDS, have not yet published any guidelines for either risperidone or aripiprazole. In the near future, it is expected that other PGx working groups will publish guidelines to facilitate the translation of risperidone/aripiprazole PGx into routine clinical practice (Biswas et al., 2022b). Our group assigned a *CYP2D6* score of '1' as NM in risperidone due to the comparative blood concentration levels of risperidone. However, a *CYP2D6* score of 'I' was assigned as IM by the recent CPIC guidelines for selective serotonin reuptake inhibitors (SSRIs) instead of NM (Hongkaew et al., 2021b; Bousman et al., 2023). The controversy regarding *CYP2D6* scoring systems and predicted phenotypes for risperidone metabolism that has arisen between "the consortium (CPIC + DPWG)" and "the PPM-pharmacogenomics of autism spectrum disorders of the Thailand Project" is shown in Table 3. The government of Thailand should consider the *CYP2D6* scoring system for risperidone as suggested by the PPM Laboratory, since it may be highly applicable for Thai patients and may expedite the formation of prescribing guidelines, which may further help to improve the safety or effectiveness of risperidone in ASD.

12.2 Clinical implementation

Many factors, including infrastructure and robust evidence, are involved in the successful implementation of PGx in routine clinical practice. Precision medicine for ASD may be achieved through ensuring PGx screening for at least *CYP2D6* genetic variants in routine clinical practice (Biswas et al., 2022b).

12.3 Reimbursement

Often, reimbursement for genetic testing hinders the clinical implementation of PGx. Reimbursement coverage should be applied for ASD medications, or at least for risperidone, with wider clinical adoption (Biswas et al., 2022b).

12.4 Undetermined and rare SNPs

Novel genes and SNPs should be considered. WGS can help with their discovery, such as our finding of novel SNPs in the discrepancy between the risperidone level and *CYP2D6* genotyping, leading to the determination of novel **142*, **143*, and **144* in an ASD study (Hongkaew et al., 2021c).

12.5 Healthcare provider awareness and knowledge

Healthcare professionals must be aware of the PGx associations of ASD medications and, obviously, must have adequate knowledge about the PGx interference of ASD medications. Along with pharmacists, doctors are the main driving force behind the implementation of newly evolving PGx approaches in real clinical practice (Albassam et al., 2018; Edris et al., 2021; Biswas et al., 2022b). Since many of these healthcare professionals do not have sufficient knowledge or confidence to implement precision medicine in clinical settings, education and trainings are obvious to make them professionally competent. A recent study concluded that an adaptable and flexible training module is needed for targeted healthcare professionals for the successful implementation of precision medicine in routine clinical practice (Mitchell et al., 2022).

13 Conclusion

Although there is no selective pharmacotherapy for ASD, the FDA has recommended risperidone/aripiprazole to treat associated symptoms of ASD, such as agitation/irritability. Strong associations of some pharmacokinetic/pharmacodynamic gene variants, e.g., *CYP2D6* and *DRD2*, with risperidone-induced hyperprolactinemia have been found in children with ASD, but such genetic associations have not been found directly for aripiprazole in ASD. In addition to PGx factors, DDIs and possibly the cumulative effects of DDIs and PGx, called multifactorial DGIs, may regulate the safety or effectiveness of risperidone/aripiprazole, which should be assessed in future clinical studies in children with ASD. Reimbursement, knowledge, and education of healthcare professionals are the key obstacles preventing the successful implementation of ASD pharmacogenomics into routine clinical practice. The preparation of national and international PGx-based dosing guidelines for risperidone/aripiprazole based on robust evidence may advance the precision medicine of ASD.

Author contributions

MB: data curation, formal analysis, investigation, methodology, resources, writing–original draft, and writing–review and editing. NV: data curation, formal analysis, investigation, methodology, writing–original draft, and writing–review and editing. CS: conceptualization, funding acquisition, investigation, methodology, project administration, supervision, validation, visualization, and writing–review and editing.

Funding

The authors declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by the 1) Mahidol University International Postdoctoral Fellowship, Mahidol University; 2) Faculty of

Medicine, Ramathibodi Hospital, Mahidol University; and 3) Ratchadapisek Somphot Fund, Chulalongkorn University.

Acknowledgments

The authors would like to thank the staff of the Pharmacogenomic and Personalized Medicine Laboratory of Ramathibodi Hospital.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1285967/full#supplementary-material

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frontiers | Frontiers in Pharmacology

TYPE Review
PUBLISHED 14 February 2024
DOI 10.3389/fphar.2024.1326776

OPEN ACCESS

EDITED BY
Francesca Coperchini,
University of Pavia, Italy

REVIEWED BY
Otito Frances Iwuchukwu,
Fairleigh Dickinson University, United States
George P. Patrinos,
University of Patras, Greece

*CORRESPONDENCE
Maha Saber-Ayad,
msaber@sharjah.ac.ae

RECEIVED 23 October 2023
ACCEPTED 25 January 2024
PUBLISHED 14 February 2024

CITATION
Shubbar Q, Alchakee A, Issa KW, Adi AJ, Shorbagi AI and Saber-Ayad M (2024), From genes to drugs: *CYP2C19* and pharmacogenetics in clinical practice. *Front. Pharmacol.* 15:1326776. doi: 10.3389/fphar.2024.1326776

From genes to drugs: *CYP2C19* and pharmacogenetics in clinical practice

Qamar Shubbar[1,2], Aminah Alchakee[1,2], Khaled Walid Issa[1], Abdul Jabbar Adi[1], Ali Ibrahim Shorbagi[1] and Maha Saber-Ayad[1,2]*

[1]College of Medicine, University of Sharjah, Sharjah, United Arab Emirates, [2]Sharjah Institute for Medical Research, University of Sharjah, Sharjah, United Arab Emirates

The *CYP2C19* gene is frequently included in different pharmacogenomic panels tested in clinical practice, due to its involvement in the metabolism of a myriad of frequently prescribed medications. Accordingly, *CYP2C19* genotyping can promote precise therapeutic decisions and avoid the occurrence of significant drug-drug-gene interactions in the clinical setting. A comprehensive examination of the role of the *CYP2C19* gene in real-world medical settings is presented in this review. This review summarizes the most recent information on how genetic variants in *CYP2C19* affect drug metabolism and therapeutic outcomes. It goes into the wide range of CYP2C19 phenotypes, with different degrees of metabolizing activity, and their implications for customized medication response through a review of the literature. The review also analyzes the clinical significance of *CYP2C19* in several medical specialties, including cardiology, psychiatry, and gastro-enterology clinics, and illuminates how it affects pharmacological efficacy, safety, and adverse effects. Finally, *CYP2C19*-supported clinical decision-making is outlined, highlighting the possibility of improving therapeutic outcomes and achieving more affordable treatment options, a step towards optimizing healthcare provision through precision medicine.

KEYWORDS

***CYP2C19*, pharmacogenetics, precision medicine, gentotype, pharmacoeconomic, CPIC, ethnic variation, gene polymorphism**

1 Introduction

With the advancements in pharmacogenetics, *CYP2C19* has emerged as a gene for personalized drug prescriptions that serve many medical specialties, by considering the effect of genetic variants on the expected drug response (Naujokaitis et al., 2021). Therapeutic guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC) are commonly used to recommend an appropriate treatment regimen based on the genotype test, specifically for patients in need of antiplatelet medication (Lee et al., 2022). Understanding the relevance of *CYP2C19* is essential for realizing the full promise of personalized medicine to improve medication use and transform contemporary healthcare (Zanger and Schwab, 2013). The insight gained from the diverse genetic alterations in *CYP2C19* and their consequences on enzyme action has ushered in a significant transformation in the approach to drug prescription and distribution. Healthcare professionals can maximize treatment efficacy while lowering the risk of adverse medication responses by implementing pharmacogenomic testing (Lee, 2013). This

TABLE 1 Drugs primarily metabolized by *CYP2C19*. Data obtained from PharmGKB.

Drugs primarily metabolized by *CYP2C19*
Gastrointestinal agents
Lansoprazole
Pantoprazole
Omeprazole (Esomeprazole)
Anti-infective agents
Voriconazole
Neurological and psychiatric agents
Benzodiazepine
Citalopram and Escitalopram
Clobazam
Clomipramine
Sertraline
Fluoxetine
Imipramine
Trimipramine
Doxepin
Cardiovascular and hematology agents
Clopidogrel
Pain and Anti-inflammatory agents
Cannabidiol
Drugs secondarily metabolized by *CYP2C19*
Psychiatric agents
Venlafaxine
Amitriptyline and Nortriptyline
Cardiovascular agents
Rosuvastatin
Neurological agents (with minor involvement of CYP2C19)
Clozapine
Paroxetine
Phenytoin
Venlafaxine
Analgesic and Anti-inflammatory agents
Methadone

article will explore the practical applications of *CYP2C19* genotyping, particularly in handling cardiovascular (such as Clopidogrel), psychiatric (such as sertraline, fluoxetine, citalopram, escitalopram, and others), and gastrointestinal (such as Pantoprazole, Lansoprazole and Omeprazole (Esomeprazole)) conditions and drugs. The review aims to assess the role and impact of CYP2C19 status in current clinical settings. Furthermore, the challenges and benefits of integrating *CYP2C19* genotyping into healthcare strategies are discussed, considering elements such as cost-efficiency and prospective advances in personalized medicine. By meticulously analyzing both established literature and recent studies, this review seeks to contribute to the growing compendium of knowledge regarding the assimilation of *CYP2C19* genotyping into routine clinical practice.

2 Methodology of searching

Q.S. and Am.A. conducted an extensive search of PubMed and MEDLINE databases, covering literature from 2010 until October 2023. They independently screened titles, abstracts, and full texts to assess the eligibility of articles. The search methodology adhered to the Preferred Reporting Items for Systematic Reviews and Review Articles. The number of results was 44,613. However, after excluding review articles, animal studies and case reports, as well as duplications of data, they reached to the number of references used in this review article. The search strategy included the following relevant terms: "pharmacogenomics AND CYP2C19", "clinical practice AND CYP2C19", "ethnic variation AND CYP2C19", "cardiology AND CYP2C19", "psychiatry AND CYP2C19, "gastroenterology AND CYP2C19". The inclusion criteria were English language, clinical trials, observational studies, pharmacokinetics studies, and epidemiological studies.

3 *CYP2C19* gene, and the role of CYP2C19 as a drug-metabolizing enzyme

The CYP450 superfamily is a large and diverse group of enzymes whose main function is to metabolize many drugs. CYP2C19 is a member of the CYP2C subfamily of cytochromes which are involved in the metabolism of a range of clinically important compounds, such as anticoagulants, proton pump inhibitors (PPIs), benzodiazepines, anticonvulsants, and tricyclic antidepressants (Saeed and Mayet, 2013). The drugs that are primarily metabolized by the CYP2C19 enzyme are listed in Table 1. The *CYP2C19* gene is located on chromosome 10q23.33 and to date, 39 alleles and 2000 SNPs have been identified (Shao et al., 2020). Among these variants, *CYP2C19**2 and *CYP2C19**3 are the most frequent and have received the greatest attention, as they identify poor metabolizers (Ionova et al., 2020) Understanding CYP2C19 as a metabolizing enzyme will enable healthcare professionals to make decisions regarding drug selection and dosing based on each individual's genetic makeup to reach a safe and effective treatment (Pierre-François et al., 2022). Intriguingly, the *CYP2C19* gene is highly polymorphic, leading to changes in enzymatic activity, therapeutic responses, and/or adverse drug reactions. Within the CPIC guidelines, the system used to translate genotype to phenotype depends on the star (*) allele nomenclature (Botton et al., 2021). In particular, an individual is categorized as a normal [previously described as extensive metabolizer (EM)], intermediate metabolizer (IM), poor metabolizer (PM), rapid metabolizer (RM), or ultra-rapid

TABLE 2 Comparing different methods of *CYP2C19* testing.

Method	Description	Time	Sample type	Cost	Available Kit/Assay (Company)
Genotyping (PCR-based test), some will require subsequent DNA/Sanger sequencing	Identifies specific *CYP2C19* alleles using polymerase chain reaction	1–3 days	Blood	$357 to $1,230	TaqMan™ SNP Genotyping Assay, human (Thermo Fisher Scientific)
				-	xTAG® *CYP2C19* Kit v3 (Luminex)
				-	gb PHARM *CYP2C19* (Generi Biotech)
				-	*CYP2C19* Genotyping Diagnostic Kit (PCR-Fluorescence Probing) (DaAnGene)
				-	Mutector™ *CYP2C19* Genotyping kit (TrimGen)
DNA sequencing	Comprehensive analysis of the entire *CYP2C19* gene	1–2 weeks		High	-
NGS (Next-generation sequencing)	High-throughput DNA sequencing to analyze multiple genes	1–2 weeks		High	-
Point-of-Care Testing	Rapid on-site testing for immediate results in emergency settings	Few hours		Moderate	INFINITI *CYP2C19* Assay (AutoGenomics)

metabolizer (UM) based on CYP2C19 metabolizing activity which is determined by their genetic profile. The most prevalent phenotype is the normal metabolizer with *CYP2C19*1* genotype, in which individuals would be predicted to have full CYP2C19 functioning enzyme, allowing them to metabolize medicines efficiently. Poor metabolizers (PM) with *CYP2C19*2* or *CYP2C19*3* genotypes have restricted or absent CYP2C19 enzymatic activity, resulting in a delay in drug metabolism and probable drug toxicity, which can lead to unpleasant adverse effects. Intermediate metabolizers (IM) carry one loss-of-function allele such as the genotype *1/*2, allowing for intermediate drug metabolism. Ultra-rapid metabolizers (UM) with the *CYP2C19*17* genotype, on the other hand, have overactive variations of the CYP2C19 gene, resulting in faster medication metabolism and clearance. Patients with ultra-rapid metabolizer phenotype may require higher doses of drugs to reach the intended therapeutic response (Rollinson et al., 2020).

4 *CYP2C19* testing

Pharmacogenomics (PGx) testing can involve a single gene or a panel of multiple genes. Evaluations of commercial PGx testing panels have revealed that gene composition as well as variant composition varies from test to test and frequently contains genes for which evidence is lacking to recommend prescription in various clinics, besides the fact that the *CYP2C19* gene is so large that, whole gene cannot be routinely performed. As a result, the number of genes on a testing panel is insufficient as a criterion for test selection. Even though the same genes appear on a testing panel, the number of sequence variants, or alleles, tested within those genes might vary significantly between tests. To achieve a high level of analytical validity (i.e., the capacity of a test to identify whether a certain genetic variation is present or missing), PGx testing should preferably be done in laboratories that have been certified and accredited under national regulations. According to the Clinical Laboratory Improvement Amendments (CLIA) and the College of American Pathologists (CAP), a validation process must be carried out to evaluate the accuracy, precision, reference interval, sensitivity, and specificity of a test (Black et al., 2020) When a person undergoes *CYP2C19* genetic testing, the analysis aims to identify and characterize specific genetic variations or alleles within the *CYP2C19* gene. The *CYP2C19* gene can have different forms, known as alleles, due to genetic variations among individuals. *CYP2C19* testing provides specific alleles. Different methods and approaches for *CYP2C19* testing are shown in Table 2.

5 *CYP2C19* genotype-guided therapy (individualized prescription)

Genotype-guided treatment, also known as tailored prescription, is an advanced strategy in personalized medicine that optimizes drug selection and administration based on a patient's exact genetic composition. By evaluating genetic variations of the *CYP2C19* gene, healthcare practitioners can determine an individual's drug metabolism phenotype and classify them as normal metabolizer (NM), poor metabolizer (PM), intermediate metabolizer (IM), rapid metabolizer (RM) or ultra-rapid metabolizer (UM) (El Rouby et al., 2018)Clinicians may adjust medicine prescriptions based on a patient's specific metabolic profile, ensuring that patients receive the most effective treatment, safely. Genotype-guided treatment is very useful when prescribing drugs with proven *CYP2C19* involvement, such as clopidogrel for cardiovascular illnesses or PPIs for digestive disorders. *CYP2C19* genotype-guided drug prescribing has been proven in several trials to increase pharmacological effectiveness and safety. For example, CYP2C19 PMs have been observed to have a greater risk of cardiovascular events in patients receiving antiplatelet treatment

TABLE 3 *CYP2C19* genotype-guided therapy in cardiology practice.

Study/Clinical trial	Number of Participants	Condition	Findings	Therapy received	References
Diverse Clinical Settings	3,342	Percutaneous Coronary Intervention	CYP2C19 LOF carriers treated with alternative therapy had lower atherothrombotic risk compared to clopidogrel and similar risk in those with non-LOF allele treated either with clopidogrel or alternative therapy	Alternative therapy with prasugrel or ticagrelor compared to Clopidogrel	Beitelshees et al. (2022)
Single-center observational cohort study (China)	1,361	Percutaneous coronary intervention	The MACCE rate was higher in the LOF-clopidogrel group compared with the LOF-ticagrelor group ($p = 0.029$). No significant difference in the incidence of MACCE, in non-LOF-clopidogrel compared to LOF-ticagrelor group ($p = 0.272$)	Ticagrelor compared to Clopidogrel	Zhang et al. (2021)
TAILOR-PCI Randomized Clinical Trial (US, Canada, South Korea, and Mexico)	5,302	Percutaneous coronary intervention (for acute coronary syndromes (ACS) or stable coronary artery disease (CAD))	Genotype-guided therapy of P2Y12 inhibitor, compared with conventional clopidogrel therapy, showed no statistically significant difference	Ticagrelor and noncarriers clopidogrel compared to Clopidogrel	Pereira et al. (2020)
Real-world cohort (University of North Carolina-Chapel Hill)	1,063	Percutaneous Coronary Intervention	Compared to alternative therapy, CYP2C19 LOF allele carriers receiving clopidogrel showed a significantly higher risk of MACCE or bleeding over 30 days	Genotype-guided prescribing, ticagrelor or prasugrel	Williams et al. (2019)
Single-center, non-randomized, retrospective cohort study (China)	1,134	Off-pump coronary artery bypass grafting (OPCAB)	individual DAPT (CYP2C19 genotype with platelet aggregation test) was associated with lower risk of MACE and a similar risk of major bleeding compared to traditional DAPT (Aspirin with Clopidogrel)	Dual antiplatelet therapy (DAPT)	Yao et al. (2022)
Prospective, open-label RCT	650	Ischemic stroke or transient ischemic attack (TIA)	Clopidogrel guided therapy can significantly improve the overall clinical benefit of ischemic stroke or TIA patients without increasing the risk of bleeding	The pharmacogenetic group received aspirin combined with clopidogrel/ticagrelor based on clinical characteristics. While the standard group received aspirin combined with clopidogrel	Zhang et al. (2023)

with clopidogrel following stent installation. Switching PMs to alternative antiplatelet medications, such as ticagrelor or prasugrel, based on their genotype, has been associated with better clinical outcomes (Castrichini et al., 2023). A summary of studies on *CYP2C19* Genotype-Guided therapy in cardiovascular practice is shown in Table 3. *CYP2C19* genotype-guided medication has the potential to increase treatment efficacy, minimize adverse drug responses, and improve overall patient outcomes by incorporating genetic information into the treatment decision-making process, ushering in a new age of personalized medicine. Other aspects, such as drug interactions, co-existing illnesses, and lifestyle issues, must also be considered to achieve thorough and holistic patient treatment.

6 *CYP2C19* in clinical practice

6.1 *CYP2C19* in cardiology

i. Significant recommendations in clinical practice according to the gene variant:

CYP2C19 gene variants play an important role in cardiology, primarily considering the metabolism of commonly used medications. These gene variants can significantly influence how patients metabolize drugs, especially, clopidogrel, a widely used antiplatelet drug (Pereira et al., 2019). Poor metabolizers may exhibit reduced conversion of clopidogrel to its active form, thus compromising its effectiveness in preventing

TABLE 4 Dosing recommendations for clopidogrel based on *CYP2C19* phenotype. Data with strong evidence was retrieved from CPIC guidelines.

Phenotype	Drug	Haplotype	Result	Therapeutic recommendation	Classification	References
Ultrarapid metabolizers	Clopidogrel	*17/*17	Due to increased metabolism, it will result in a lower antiplatelet reactivity, and no association with bleeding risk	If using Clopidogrel, use the standard dose of 75 mg	Strong	Lee et al. (2022)
Rapid metabolizer		*1/*17	Normal or increased metabolism of the drug, and no association of bleeding risk			
Intermediate metabolizer		*1/*9, *9/*17, *9/*9	Due to reduced metabolism, lower platelet reactivity will result, increasing the risk of cardiovascular events	Avoid the standard dose of Clopidogrel and use Prasugrel or Ticagrelor at standard dose if no contraindication	Moderate	
Poor metabolizer		*2/*9, *3/*9	Significantly reduced clopidogrel metabolism, increased risk of cardiovascular risk	Avoid Clopidogrel and if no contraindication, use Prasugrel or Ticagrelor		

cardiovascular events. In consideration of this, cardiology practice recommendations emphasize the importance of genotyping *CYP2C19* variants to identify patients at higher risk and personalize their medications accordingly (Turner and Pirmohamed, 2014). On the other hand, concomitant use of clopidogrel and PPIs may reduce the activation and it will affect the efficacy of clopidogrel, hence the need for clinicians to consider PPIs with minimal *CYP2C19* dependent (Kenngott et al., 2010). Table 4 summarizes *CYP2C19* gene variation based on the following recommendations that are relevant in the cardiology clinic.

ii. Recommended panels:

When considering whether to include *CYP2C19* genotyping with other genes in the cardiology clinic, it is vital to concentrate on genes that can alter how drugs are metabolized, especially for medications that are often prescribed. Together with *CYP2C19*, several genes may be taken into consideration for panels, such as *CYP2C9*, another essential gene in drug metabolism that is particularly important for drugs like warfarin and non-vitamin K oral anticoagulants (NOACs). *VKORC1* is another crucial gene, as it influences the dose needed to achieve the desired anticoagulation effect of warfarin. Combining *CYP2C19*, *CYP2C9*, and *VKORC1* genotyping helps researchers to have a full picture of how patients metabolize these drugs (De Lara et al., 2022). The *ADRB1* gene, which encodes for the beta-1 adrenergic receptor, is one of the genes that have an impact on the response of beta blockers which are commonly used in the treatment of heart failure and hypertension. Considering patients' *ADRB1* genotype will provide clinicians with guidance for the selection and dose of these medications (Howaidi and Lababidi, 2022). The *ADRA2A* gene which encodes for alpha-2A receptor, has a vital role in the regulation of the sympathetic nervous system. Variation in the *ADRA2A* gene can also influence the patient's response to beta-blocker drugs. The precision of cardiovascular therapy could be improved by including these genes in a curated panel as it would enable a more individualized approach that considers a person's genetic variables, lowering the chance of adverse reactions and ensuring that patients receive the best possible care.

iii. Adverse effects:

In a study on 168 patients with coronary heart disease who received clopidogrel/dual antiplatelet therapy (DAPT) after percutaneous coronary intervention (PCI), the incidence of cardiovascular adverse events was recorded by the high-on treatment-platelet reactivity (HPR) at 1-year follow-up visits. HPR was measured using thrombo-elastography which is a test used to assess the efficiency of blood coagulation. Moreover, PCR was done at the beginning of the study to determine *CYP2C19* and *ABCB1* $3435^{-\Delta\Delta CT}$ gene polymorphisms. The study concluded that the non-functional *CYP2C19**3 variant was associated with a higher incidence of HPR which was correlated with a higher incidence of cardiovascular adverse events. On the other hand, the non-functional allele *CYP2C19**2 and *ABCB1* $3435^{-\Delta\Delta CT}$ were not significantly associated with HPR or cardiovascular events. This suggests that *CYP2C19* and *ABCB1* $3435^{-\Delta\Delta CT}$ genotyping before initiation of clopidogrel therapy can be a significant predictive factor for treatment failure and the development of adverse effects (Mega et al., 2010). DAPT with aspirin and a P2Y12 inhibitor (clopidogrel, prasugrel, or ticagrelor) is known to be the standard care therapy for patients following PCI. The CYP2C19 enzyme is responsible for the activation of the prodrug "clopidogrel" into its active metabolite to carry out its antiplatelet activity. Patients who carry the non-functioning allele of *CYP2C19* and receive clopidogrel as anti-platelet therapy are at a higher risk of treatment failure and development of major adverse cardiovascular and cerebrovascular events. Prasugrel and ticagrelor have not been linked with *CYP2C19* activation like clopidogrel, which makes them better options for patients with non-functioning *CYP2C19* alleles. Therefore, *CYP2C19* genotype-guided anti-platelet therapy is believed to be beneficial for the prevention of major adverse cardiovascular and cerebrovascular events, which is further reinforced by multiple data emerging from

cardiovascular and neurology clinical studies (Sanderson et al., 2005). Multi-factorial drug-gene interaction is the umbrella term used to describe the cumulative effects of both drug-drug interactions and drug-gene interactions. This phenomenon can be applied to patients who inherited the *CYP2C19* loss-of-function allele and are receiving clopidogrel with concomitant PPI administration. In a systematic review and meta-analysis, five studies were included, comprising 8,802 patients of coronary heart disease or stroke. 3,767 were prescribed clopidogrel alone, 1,931 were concomitantly taking clopidogrel and PPIs, 2,146 were carrying *CYP2C19* loss-of-function alleles and 958 were taking both clopidogrel and PPIs while also carrying *CYP2C19* loss-of-function alleles. Patients with coronary heart disease or stroke who are receiving clopidogrel and concomitant proton pump inhibitor (PPI) therapy while inheriting loss-of-function alleles (*CYP2C19*2* or *CYP2C19*3*) had a 63% higher risk of developing major cardiovascular adverse events (Biswas et al., 2021). In another systematic review and meta-analysis, 12.2% carried the *CYP2C9*2* variant and 7.9% carried the *CYP2C9*3* variant. Previous reports showed a 17% reduction in the original warfarin dose for *CYP2C9*2* carriers and 37% for *CYP2C9*3* carriers with a relative bleeding risk of 1.91 and 1.77, respectively. The study concluded that patients who carry *CYP2C9*2* and *CYP2C9*3* have a lower mean daily warfarin dose and a higher bleeding risk, which speculates that genotype-guided warfarin therapy could markedly alter the management of patients being started on warfarin (Barbarino et al., 2018). However, it showed that there is a marked difference in the enzymatic activity between *CYP2C9*2* and *CYP2C9*3* carriers. Poor metabolizer patients on Clopidogrel carrying CYP2C19 *3/*9 genotype may experience diminished antiplatelet effects, potentially increasing the risk of cardiovascular events, thus, they may use alternative drugs such as Prasugrel or Ticagrelor. The diplotype is more commonly seen in African American/Afro-Caribbean and Sub-Saharan African populations as the frequency is 0.01% according to CPIC, while in Latino population its frequency is 0.0001%. Such meta-analyses signify the critical role of *CYP2C19* as a key pharmacogene in cardiology practice.

iv. Drug-drug interactions:

Co-occurrence of drug-drug interaction (DDI) with drug-gene interaction (DGI) might alter drug biotransformation pathways and produce drug-drug-gene-interaction (DDGI). PPIs are commonly used in the treatment of gastric disorders. Omeprazole is among the PPIs which poses the highest propensity to interact with other drugs compared to other PPIs like pantoprazole, rabeprazole, and lansoprazole. This is explained by its high affinity for CYP2C19 and moderate affinity for CYP3A4. Studies published since 2006 have shown clinically significant interaction between the antiplatelet medication, clopidogrel, and omeprazole which is mediated by CYP2C19 (Barbarino et al., 2018). Patients receiving DAPT often receive PPI therapy to reduce the risk of bleeding. Clopidogrel, the antiplatelet agent most commonly used in DAPT poses a challenge when concomitant PPI therapy is given due to their conflicting pharmacokinetic interaction *via* CYP2C19. Even though concomitant use of PPI and DAPT has been shown to decrease active metabolites of clopidogrel and ex vivo-measured platelet inhibition, there is still a conflict about whether this interaction has a significant effect on clinical outcomes (Saven et al., 2022).

6.2 *CYP2C19* in psychiatry

i. Significant recommendations in clinical practice according to the gene variant:

The CYP2C19 enzyme plays a crucial role in the metabolism of many antidepressants, including selective serotonin reuptake inhibitors (SSRIs) such as sertraline, fluoxetine, citalopram, escitalopram, and others. In addition, multiple conventional tricyclic antidepressants such as imipramine, amitriptyline, trimipramine and clomipramine, are known CYP2C19 substrates (Alchakee et al., 2022). Genetic variations of *CYP2C19* significantly impact the efficacy and safety of antidepressant medications, thus clinically influencing depression management. Table 5 summarizes dosing recommendations of antidepressants classified as level 1A evidence. Level 1A evidence indicates a specific gene-variant prescribing advice is provided in current clinical guidelines or FDA-approved drug label annotations (Hicks et al., 2015). Based on the *CYP2C19* genotype, the CPIC published gene-based therapy recommendations for the SSRIs citalopram and escitalopram. For CYP2C19 ultrarapid and poor metabolizers, it is recommended to use an alternative antidepressant that is not primarily metabolized by CYP2C19 or to adjust the dose according to metabolizer status. Furthermore, people with a *CYP2C19* *17/*17 genotype have significantly lower citalopram or escitalopram plasma concentrations at steady state when compared to normal metabolizers, thus it is recommended to titer citalopram to a higher target dose (compared to normal metabolizers) or to initiate an alternative SSRI, such as fluoxetine, fluvoxamine, and paroxetine, which are strongly metabolized by CYP2D6 only (Wong et al., 2023). The choice of an alternative antidepressant medication should be individualized based on the patient's specific needs and medical history.

ii. Recommended panels:

The cytochrome P450 isoenzymes, mainly CYP2D6, CYP2C9, and CYP2C19 are responsible for the metabolism of the majority of psychotropic medications, including antipsychotics, antidepressants, and mood stabilizers. The highly polymorphic CYP2C19 enzyme plays a crucial role in the metabolism of many antidepressants, including SSRIs such as, sertraline, fluoxetine and (es)citalopram. Psychiatric gene sequencing panels vary depending on the specific focus of a clinic, patient population, psychiatric disorders, or medications of interest (Thiele et al., 2022). CYP2C19 demethylates several tricyclic antidepressants including, clomipramine, amitriptyline, trimipramine and imipramine to pharmacologically active metabolites. These compounds and their metabolites along with nortriptyline and desipramine, are hydroxylated by CYP2C19 enzyme to fewer active metabolites (Hicks et al., 2013). Therefore, combining *CYP2D6* and *CYP2C19* genomic variants in a single panel can provide a more

TABLE 5 Dosing recommendations for antidepressants based on *CYP2C19* phenotype. Data with strong evidence were retrieved from PharmGKB and CPIC guidelines.

Drug name	Genotype	Phenotype	Dose recommendation	Alternative Antidepressant
SSRI				
Es-/citalopram	*17/*17	Ultra-rapid	consider titrating to a higher maintenance dose	Fluoxetine, Fluvoxamine, Paroxetine. (Nikolac Perkovic et al., 2020)
	*2/*9, *3/*9	Likely Poor Metabolizer	Per the FDA warning, citalopram 20 mg/day is the maximum recommended dose in CYP2C19 poor metabolizers due to the risk of QT prolongation	
	*2/*2, *2/*3, *3/*3	Poor metabolizers	citalopram dose should be limited to 20 mg/day in patients with hepatic impairment, those taking a CYP2C19 inhibitor, and patients greater than 60 years of age	
Sertraline	*17/*17	Ultra-rapid	Initiate therapy with the recommended starting dose	
	*1/*17	Rapid Metabolizer	Initiate therapy with the recommended starting dose	
Tricyclic Antidepressants (TCAs)				
Amitriptyline	*1/*2, *1/*3, *2/*17	Intermediate metabolizer	Initiate therapy with the recommended starting dose	Bupropion, Fluvoxamine, Mirtazapine or Paroxetine (Kee et al., 2023)
Clomipramine	*1/*1	Normal metabolizer	Initiate therapy with the recommended starting dose	
Imipramine				
Trimipramine				

comprehensive understanding of an individual's drug metabolism profile that affects drug efficacy and safety (Matthaei et al., 2021). Interestingly, in psychiatry, *ADRB1* polymorphisms, along with other genes, increase the risk of developing Alzheimer's disease and sleep disturbances caused by altered cell responsiveness to adrenergic stimulation (Bullido et al., 2004). Genetic variations of the catechol-O-methyltransferase (*COMT*) gene, which encodes for an enzyme involved in the metabolism of dopamine and norepinephrine, have been associated with altered response to antipsychotic medications (Nikolac Perkovic et al., 2020). Notably, some studies in cardiology have explored a relationship between *COMT* variants and hypertension; however, data on this is scarce, and further investigations are required (Xu et al., 2017). In general, combining *CYP2C19* with other actionable pharmacogenes in a genotyping test can provide valuable insights into personalized medicine.

iii. Adverse effects:

The discontinuation of antidepressant treatment is a common behavior in people with depression, mainly due to adverse drug reactions. Nearly 50% of undesirable drug reactions can be attributed to the differences in drug metabolism between individuals (Solomon et al., 2019; Kee et al., 2023). A clinical study was conducted to explore the association of *CYP2C19* actionable variants translated into phenotypes with suicidal behavior in patients with depression who were using citalopram. The rate of suicide was 2-fold higher in individuals classified as CYP2C19 poor metabolizers compared to those classified as CYP2C19 normal metabolizers (Aldrich et al., 2019; Joas et al., 2023). The association of CYP2C19 metabolism status and side effects including hyperactivity, weight gain, gastrointestinal symptoms and insomnia was also investigated in pediatric patients prescribed escitalopram for anxiety or depressive disorders. The CYP2C19 poor metabolizers experienced more unwanted effects compared to faster metabolizers. In particular, CYP2C19 PMs had more rapid weight gain and hyperactivity (Ramsey, 2018). A recent Australian study consisting of 9,500 participants revealed that escitalopram is more tolerable by rapid CYP2C19 metabolizers while sertraline is more tolerable by poor CYP2C19 metabolizers, compared to normal metabolizers (Campos et al., 2022). On the other hand, a Swedish genetic study has revealed that the incidence of treatment-emergent mania was increased in patients with slower CYP2C19 metabolism status who were using amitriptyline or sertraline to treat bipolar depression with a hazard ratio (1.3, 1.46), respectively (Rahikainen et al., 2019). The Pre-emptive Pharmacogenomic Testing for Preventing Adverse Drug Reactions (PREPARE) study is the first large-scale and randomized clinical trial conducted in Europe to investigate the impact of applying pharmacogenomic test on the incidence of adverse drug reactions. The PREPARE study covered 39 different medications to treat multiple diseases. Notably, preemptively tested participants with actionable variants experienced a remarkable 30% reduction in the incidence rate of clinically relevant adverse drug reactions associated with drug-genotype interactions (Swen et al., 2023). Therefore, determining the patient's CYP2C19 metabolizing status based on his genetic profile might enhance the safety of using antidepressant medications (Joas et al., 2023).

iv. Drug-drug interactions:

As previously mentioned, significantly altered rates of metabolism may occur due to DDGI. Escitalopram is mainly metabolized by the CYP2C19 and CYP3A4 enzymes and to a lesser extent by the CYP2D6 enzyme. Blood concentration of escitalopram is significantly influenced by the concomitant administration of *CYP2C19*, *CYP3A4*, and *CYP2D6* modulator drugs (Rochat et al., 1997). The Combination of *CYP3A5* and *CYP2C19* genetic

TABLE 6 Recommendations based on *CYP2C19* gene variants in the Gastroenterology clinic.

Phenotype	Drug	Reason of using the drug	Haplotype	Result	Alternative	References
Poor metabolizers	Omeprazole/ Pantoprazole	Different PPI's indications	*2/*2, *2/*3, *3/*3	Due to decreased metabolism, the efficacy will be reduced	Rabeprazole, which is less dependent PPI on *CYP2C19*	Lima et al. (2021)
	Clopidogrel	Cardiovascular patients with gastrointestinal issues		Reduced effectiveness of Clopidogrel and increase in cardiovascular adverse effects	Ticagrelor or Prasugrel, are used as they are not influenced by *CYP2C19*	Lee et al. (2022)
	Escitalopram	Manage some gastroenterological conditions associated with anxiety or depression		Higher drug levels, so increased risk of side effects	Dosing adjustments or consider alternative antidepressant medications	Bousman et al. (2023)

polymorphisms mediates several DDIs and DGIs. For instance, the co-presence of CYP3A4 EM and CYP2C19 IM/PM increases the risk of (es)citalopram toxicity and hence the urge for dose reduction or drug switching (Bahar et al., 2020). Unfortunately, the recent dosage recommendation for escitalopram is based on DGIs and DDIs separately and a knowledge gap remains regarding In a recent clinical case report, a patient complained of inadequate depression control despite several attempts with multiple antidepressants, including escitalopram, venlafaxine, and bupropion. The patient was phenotypically a *CYP2C19* IM and a *CYP2D6* PM (due to phenoconversion), and genetic variants in *CYP2D6* and *CYP2C19* increased their venlafaxine plasma concentration. In addition, the metabolism of other concomitant medications was impacted by the strong *CYP2C19* inhibitor, bupropion, which contributed to the treatment failure. Cannabidiol (CBD) and PPIs are clinically known as *CYP2C19* inhibitors and hence cause *CYP2C19* phenoconversion (Von Moltke et al., 2001; Bousman et al., 2023). In particular, the concomitant use of CYP2C19 inhibitors and psychiatric medications may commonly lead to phenotype conversion from nonpoor metabolizer phenotype to poor metabolizer phenotype (Klieber et al., 2015). In a recently published case report, a patient with intermediate CYP2C19 phenotype who was on sertraline for 20 years developed cognitive dysfunction and hyponatremia due to an increase in sertraline plasma concertation after addition of CBD to their treatment regimen (Nanan et al., 2022). In another clinical case report, a patient complained of inadequate depression control despite several attempts with multiple antidepressants, including escitalopram, venlafaxine, and bupropion. The patient was phenotypically a CYP2C19 IM and a CYP2D6 PM, and genetic variants in *CYP2D6* and *CYP2C19* increased his venlafaxine plasma concentration. In addition, the metabolism of other concomitant medications was impacted by the strong CYP2C19 inhibitor, bupropion, which contributed to the treatment failure (Nanan et al., 2022).

6.3 *CYP2C19* in gastroenterology

i. Significant recommendations in clinical practice according to the gene variant:

Based on the previously described *CYP2C19* gene variation, recommendations relevant to Gastroenterology are summarized in Table 6. Individual patient variables, pharmacological interactions, and the exact clinical circumstance should all be considered when deciding on the best treatment approach. PPI dosing recommendations based on CYP2C19 phenotype reflect a prototypical integration of pharmacogenomics into gastroenterology as shown in Table 7. CYP2C19 is a key enzyme in PPI metabolism, *CYP2C19* genetic variations have important implications for the therapeutic response and safety of PPI regimens. Substantial data compiled by impactful sources such as PharmGKB (Pharmacogenomics Knowledgebase) and the stringent clinical directions provided by the CPIC guidelines contribute to the cogency of these suggestions.

ii. Recommended panels:

Genetic testing panels at a gastrointestinal clinic can offer useful information about patients' genetic tendencies and probable drug reactions. Genetic testing can be used to personalize therapy regimens, forecast illness risk, and spot potential negative effects. Although several genetic testing panels could be taken into consideration, advice on the use of particular panels may vary depending on the clinic's specialty and resources. The Inflammatory Bowel Disease (IBD) Panel, which includes *NOD2*, *IL23R*, and *ATG16L1* genes along with *CYP2C19*, which are associated with IBD severity and susceptibility can aid in defining genetic risk and tailoring treatment for patients suffering from Crohn's disease and ulcerative colitis (Slavin et al., 2019). The Liver Disease and Drug Metabolism Panel which includes *CYP2C19*, *UGT1A1*, *HFE*, and other genes, covers genetic variables impacting liver disorders and drug metabolism, which are relevant in gastrointestinal (Liu et al., 2022) and could be very relevant to clinical practice due to the liver's crucial function in digestion and drug processing. A collaborative approach could provide an understanding of the intricate genetic framework that underpins gastrointestinal wellbeing, enabling healthcare professionals to define patient-focused alignment of diagnostic and therapeutic strategies.

iii. Adverse effects:

Adverse effects pose a significant concern in the Gastroenterology clinic, and understanding both genophenotypic

TABLE 7 Dosing recommendations for Proton Pump Inhibitors based on *CYP2C19* phenotype. Data with strong evidence were retrieved from PharmGKB and CPIC guidelines.

Drug name	Diplotype	phenotype	Dose recommendation
Omeprazole, Lansoprazole, Pantoprazole, and Dexlansoprazole	*17/*17	Ultra-rapid Metabolizer	Increase starting daily dose by 100%
	*1/*17	Rapid Metabolizer	Initiate standard starting daily dose. Consider increasing the dose by 50%–100% for the treatment of *Helicobacter pylori* infection and erosive esophagitis
	*1/*1	Normal metabolizer	Initiate standard starting daily dose
	*1/*2, *1/*3, *2/*17, *3/*17	Intermediate Metabolizer	Initiate standard starting daily dose. For chronic therapy (>12 weeks) and efficacy achieved, consider 50% reduction in daily dose
	*2/*2, *3/*3, *2/*3	Poor metabolizer	

factors and pharmacogenomics can provide insights into the origins of adverse drug reactions (ADRs). Genophenotypic factors encompass genetic and phenotypic variations that significantly affect an individual's susceptibility to ADRs. Meanwhile, pharmacogenomics investigates how genetic variants affect medication metabolism, effectiveness, and safety (Chevalier et al., 2023). Adverse consequences of *CYP2C19* genetic variants can emerge as impaired drug metabolism and reactions to drugs routinely used in gastrointestinal diseases. The most prevalent side effect related to *CYP2C19* genetic variants is an altered response to PPIs in poor metabolizers carrying *CYP2C19* loss-of-function variants which leads to reduced efficacy of some PPIs like omeprazole and lansoprazole), due to impaired conversion to their active forms. This would result in diminished acid suppression, which will potentially impact symptom relief of gastrointestinal disorders such as peptic ulcer disease, and gastroesophageal reflux disease (GERD) (Paré et al., 2010). For patients with gastrointestinal and cardiac conditions, CYP2C19 poor metabolizers may have a reduced ability to convert clopidogrel to its active form, thus resulting in decreased antiplatelet activity, and leading to increased adverse cardiovascular events (Chen et al., 2012). Patients with known *CYP2C19* genetic variants should be actively examined by gastroenterologists for the risk of these unfavorable outcomes. To minimize these side effects and improve treatment results, genetic testing can disclose important information about a patient's metabolic profile, which can then be used to guide medication selection and dosing modifications.

7 Pharmacoeconomic (health burden) and *CYP2C19*

Pharmacoeconomic studies assessing the effect of *CYP2C19* genotype-guided treatment have garnered attention in recent years, the purpose of which is to analyze the economic consequences of using pharmacogenetic tests to guide pharmacological therapy selections based on *CYP2C19* genetic variations. Pharmacoeconomic studies evaluate the economic effect of various treatment regimens by considering both direct hospital expenses and larger social costs associated with illness management (Sorich et al., 2013). In a meta-analysis of pharmacoeconomic research on *CYP2C19* genotype-guided antiplatelet medication in patients with acute coronary syndrome, researchers discovered that genotyping individuals and tailoring antiplatelet medication based on *CYP2C19* variations resulted in significant reductions in severe adverse cardiovascular events and total healthcare expenditures (Fu et al., 2019). Lee et al. assessed the cost-effectiveness of *CYP2C19* genotype-guided antiplatelet treatment in patients undergoing PCI. The study found that genotype-guided medicine was a more cost-effective choice than the standard treatment, particularly in those at high risk for adverse cardiovascular events. The study highlighted the significance of incorporating pharmacogenetic testing into standard clinical practice to improve treatment results and resource use (Lee et al., 2011). Another research on the cost-effectiveness of *CYP2C19* genotype-guided antiplatelet treatment in Korean patients having PCI for acute coronary syndrome found that adding genotyping into clinical decision-making was a cost-effective strategy that resulted in improved clinical outcomes and decreased healthcare costs when compared to standard therapy. The authors emphasized the potential for significant economic gains from genotype-guided treatment (Al-Rubaish et al., 2020). Overall, data suggests that *CYP2C19* genotype-guided therapy has the potential to improve patient outcomes and reduce healthcare costs in a range of clinical contexts, notably antiplatelet treatment for cardiovascular cases. Healthcare professionals can personalize medication therapies to optimize therapeutic advantages while avoiding adverse drug responses and treatment inefficiencies by identifying patients with distinct *CYP2C19* variations. Despite the positive data, broad implementation of pharmacogenetic testing in ordinary clinical practice remains a challenge, and further research is needed to overcome adoption obstacles and enable fair access to genotype-guided medication. As the field of pharmaco-economics gains more attention, more research and real-world data will be needed to drive policy and procedure that is grounded in evidence in personalized medicine. In another research study, the authors propose that PGx-guided clopidogrel therapy is an affordable choice for ACS patients receiving care in Spain (Koufaki et al., 2023).

8 Ethnic variation

In the framework of *CYP2C19* genetics and its impact on drug metabolism, ethnic diversity is crucial to explore. Normal

metabolizers (NMs) may be more prevalent in some cultures, whilst poor metabolizers (PMs) may be more prevalent in others. Within certain ethnic groups, these variances may affect pharmaceutical reactions and efficacy. It is essential to understand ethnic diversity in *CYP2C19* genotypes to tailor pharmacological regimens and improve treatment results while taking into consideration genetic propensity and sensitivity to adverse drug responses (Nguyen et al., 2022). In a study conducted to evaluate the disparity between individuals with different racial backgrounds, when it comes to *CYP2C19* genotype-guided P2Y12 antiplatelet therapy, patients from 9 sites that performed genotyping for *CYP2C19* following percutaneous coronary intervention was recruited. A total of 3,342 participants were included, out of which 2,448 (73%) were European people and 659 (20%) were African people. The main aim was to compare the rate of prescribing P2Y12 inhibitors between European and African people races following *CYP2C19* genotyping to guide antiplatelet therapy selection after PCI. Patients who carried the non-functioning *CYP2C19* allele were prescribed alternative P2Y12 inhibitors (Prasugrel and Ticagrelor) instead of clopidogrel since clopidogrel's effectiveness would be decreased. Choosing between clopidogrel and alternative therapy based on the genotype was the primary outcome. African people had a significantly higher prevalence of carrying the non-functioning allele compared to European people. There was no statistically significant association between race (European and African people with non-functioning alleles) and the prescription of alternative antiplatelet therapy at discharge following PCI and 12 months after the last follow-up visit. According to this study, there is an absence of racial disparity in genotype-guided antiplatelet prescribing among patients receiving *CYP2C19* testing (Cavallari et al., 2023). The clinical outcomes of the coadministration of clopidogrel and omeprazole have not been adequately studied in the Asian population. It is believed that concomitant administration of omeprazole decreases the efficacy of clopidogrel due to its inhibition of the CYP450 *CYP2C19* variant, which is responsible for the activation of clopidogrel. According to several studies, this interaction has not shown an increase in mortality or incidence of myocardial infarction in Caucasians. Data are scarce regarding this combination of drugs in the Asian population, which is believed to have a high prevalence of the non-functioning allele of *CYP2C19*. In a retrospective study that utilized the medical records and prescriptions of more than12,000 Asian patients receiving clopidogrel. The study findings revealed that coadministration of clopidogrel and omeprazole had a significant positive association with the incidence of MI, but the association with mortality, cerebrovascular accidents, and coronary interventions deemed to be statistically insignificant. Additionally, there was ethnic variability, with an increased incidence of MI in the Malay and Chinese populations compared to the Indian population (Muthiah et al., 2021). East-Asian populations commonly exhibit a higher prevalence of the CYP2C19*17 allele, a genetic variant associated with increased enzymatic activity, leading to ultra-rapid drug metabolism. However, this genetic trait has implications for the use of proton pump inhibitors (PPIs). The increased enzymatic activity associated with the CYP2C1917 allele may result in faster metabolism of PPIs, potentially leading to reduced drug efficacy (Zhang, 2021). In Oceanian populations, there is a notable increase in the allele frequency of CYP2C19*2 and CYP2C19*3 genetic variants, contributing to a higher prevalence of individuals classified as poor metabolizers of clopidogrel. However, the drug's activation is heavily dependent on the enzymatic activity of CYP2C19. The *2 and *3 alleles are associated with reduced function of the CYP2C19 enzyme, leading to impaired conversion of clopidogrel into its active form (Helsby, 2016).

9 Conclusion

This review article emphasizes the importance of *CYP2C19* in clinical practice across a range of various disciplines. *CYP2C19* genotypes should be considered when prescribing drugs in Cardiology and Gastroenterology clinics, such as antiplatelet medicines and PPIs, respectively. The function of *CYP2C19* in Psychiatry and its role in individualized medicine is also significant, especially with the long-term use of psychiatry medications. As we are in the era of precision medicine, the integration of *CYP2C19* genotyping into clinical decision-making is a crucial first step toward tailoring medications to specific genetic profiles of our patients, ultimately increasing the bar for patient care.

Author contributions

QS: Data curation, Investigation, Visualization, Writing–original draft. AmA: Writing–original draft. KI: Writing–original draft. AAd: Writing–original draft. AS: Writing–original draft, Writing–review and editing. MS-A: Conceptualization, Funding acquisition, Project administration, Supervision, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. MS-A is funded by a collaborative grant provided by the University of Sharjah (Project No #2001090279).

Acknowledgments

The authors would like to acknowledge the support of the University of Sharjah.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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