

Laboratory diagnostics in personalised medicine - 36th Symposium of the Croatian society of medical biochemistry and laboratory medicine

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Highlights

- Personalised medicine tailors prevention, diagnosis, monitoring of disease progression, treatment, and treatment response to each patient
- Identifying genetic variants enables early detection and targeted therapy
- Gene-based therapies and newborn screening transform disease outcomes
- Advances in cancer genetics and pharmacogenomics guide safer and more precise care
- Laboratory diagnostics translates genomics into real clinical benefits

Abstract

Personalised medicine has become a central paradigm in modern healthcare, aiming to tailor prevention, diagnosis, and therapy to each patient's unique molecular and clinical profile. This paper provides a comprehensive overview of the lectures presented at the 36th annual Symposium of the Croatian society of medical biochemistry and laboratory medicine. It highlights key topics including the role of disease-causing variants in hereditary disorders, the development of cystic fibrosis transmembrane conductance regulator (CFTR) modulators, the evolution of newborn screening programs and spinal muscular atrophy diagnostics, emphasizing early detection and timely initiation of therapy, advances in cancer genetics, pharmacogenetics, and the translation of other laboratory-based innovations into clinical practice. Altogether, these advances reaffirm the central role of laboratory diagnostics as the cornerstone of personalised medicine, bridging genetic discovery with clinical translation and transforming modern healthcare into a predictive, preventive, and truly patient-centred discipline.

Keywords: precision medicine; genetic diseases, neoplastic syndromes; pharmacogenomics; newborn screening

Submitted: December 03, 2025

Accepted: June 04, 2026

Introduction

Personalised medicine has become a central paradigm in modern healthcare, aiming to tailor prevention, diagnosis, and treatment to each patient's unique molecular and clinical profile (1). Heredi-

tary disorders clearly illustrate this progress: detecting disease-causing variants allows precise risk assessment, presymptomatic diagnosis, and targeted therapeutic strategies (2). In terms of out-

<https://doi.org/10.11613/BM.2026.030501>

Biochem Med (Zagreb) 2026;36(3):030501

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come modifications, an excellent example is development of cystic fibrosis transmembrane conductance regulator (CFTR) modulators which shows how innovative therapies can fundamentally alter a disease’s course-turning cystic fibrosis from an inevitably fatal childhood disorder into a manageable chronic condition (3). Traditionally based on biochemical assays, newborn screening programs are now adopting genomic technologies. This shift enables presymptomatic diagnosis and treatment of conditions such as spinal muscular atrophy, where early intervention markedly improves survival and motor function (4,5). Similarly, advances in cancer genetics have revealed how germline mutations in cancer susceptibility genes are associated with cancer risk, enabling risk-appropriate screening and management recommendations, and how the synergy between germline and somatic mutations drives hereditary malignancies.

Precision oncology now applies molecular profiling to match patients with targeted therapies and innovative clinical trials (6,7). Pharmacogenomics further advances personalization by guiding therapy selection, predicting drug response and toxicity, and integrating complex drug-gene-environment interactions into routine clinical practice (8,9).

Hereditary disorders – Yesterday, today, and tomorrow

Over the past few decades, rapid advances in gene analysis technologies have transformed research

into genetic disorders-uncovering their molecular mechanisms and enabling precise diagnostic and prognostic testing. Our understanding of these conditions has deepened in parallel with advances in genetics and molecular biology. Key milestones in the development of genetic technologies are shown in Table 1. The study of hereditary disorders dates back to the 19th century, when Gregor Mendel, often regarded as the father of genetics, described the principles of inheritance through experiments on pea plants. However, a real understanding of the molecular basis of inheritance emerged in the mid-20th century. A key milestone was the discovery of deoxyribonucleic acid (DNA) structure in 1953 by James Watson and Francis Crick. This breakthrough was based on X-ray crystallography data previously obtained by Rosalind Franklin (10,11). Watson and Crick received the Nobel Prize in Physiology for this discovery. Uncovering the double-helix DNA structure made it possible to understand how genetic information is stored and transmitted. In the following decades, scientists identified numerous genes related to hereditary diseases, such as cystic fibrosis, Huntington’s disease, thalassemia, sickle cell disease, phenylketonuria, and spinal muscular atrophy. Traditional methods such as Sanger sequencing, while accurate, were slow and limited in throughput (12). At the beginning of the 21st century, a revolution in genetics came with next-generation sequencing (NGS), which allows millions of DNA fragments to be read simultaneously. This made it technically feasible and financially accessible to analyse parts

TABLE 1. Key milestones in the development of genetic technologies

Time Period	Technology / Discovery		Significance
1860s	Mendel’s laws of inheritance		Foundation of modern genetics
1953	Discovery of DNA structure	Understanding storage and transmission of genetic information	
1977	Sanger DNA sequencing	First accurate method for reading DNA sequences	
1990 – 2003	Human Genome Project	Complete mapping of the human genome	
2005 – present	NGS	Fast, cost-effective genome analysis	
From 2012	CRISPR-Cas9 genome editing	Precise genome modification; potential therapeutic applications	
2020s	gNBS, AI in genomics	Early diagnosis, personalised medicine, data-driven treatment decisions	

AI - Artificial intelligence. CRISPR-Cas9 - clustered regularly interspaced short palindromic repeats-associated protein 9. DNA - deoxyribonucleic acid. gNBS - genomic newborn screening. NGS - next-generation sequencing.

of the genome or even whole genomes. As a result, diagnosing rare hereditary diseases, discovering new genes and mutations, and understanding the genetic basis of complex disorders became much more achievable. The speed, sensitivity, and decreasing costs of these methods have made them the foundation of modern genetic diagnostics and research (13). Today, hereditary disorders are recognized as an important public health issue. People with a genomic predisposition to certain diseases, such as hereditary breast cancer, can take preventive measures early. Prenatal diagnostics and preimplantation genetic testing also allow for the detection of hereditary diseases even before birth. Gene therapy has emerged as one of the most promising areas of development, aiming to correct or replace faulty genes. New technologies, for example, clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9) offer the potential for precise genome editing but also raise important ethical questions (14). For now, treatments using these technologies are not part of routine clinical practice and remain largely within the realm of scientific research. Genomic newborn screening (gNBS) is also on the horizon, allowing for the detection of genetic variants associated with serious but potentially treatable hereditary diseases before clinical symptoms appear. While it improves diagnostic sensitivity compared to standard techniques, its implementation requires careful consideration of legal, ethical, and social issues. This includes informed consent, protection of genetic data, and especially the clinical interpretation of variants of uncertain significance (15). Looking ahead, further advancements in personalised medicine are expected, with therapies tailored to patients' individual genetic profiles. The digital analysis of large genetic datasets, combined with artificial intelligence, will help predict disease risk and guide the selection of the most effective therapies for newly diagnosed patients (16). Through real-world examples of diagnostics and treatment, this review highlights the benefits achieved through the remarkable progress in genetics over the past two decades.

Cystic fibrosis and CFTR modulators – New drugs, new disease?

Cystic fibrosis (CF) is an autosomal recessive genetic disease caused by pathogenic variants in the *CFTR* gene which encodes the CFTR chloride channel protein. This defect leads to abnormal mucus production and results in a multisystem disease, with progressive lung failure being the leading cause of mortality among CF patients. Cystic fibrosis transmembrane conductance regulator dysfunction produces thickened secretions that primarily affect the lungs but also involve the pancreas and other organs (12).

Until recently, CF therapy focused on symptomatic treatment without targeting the underlying protein defect. Symptomatic treatment, including airway clearance techniques, pancreatic enzyme replacement, and antibiotics, has significantly improved survival but could not fully prevent ongoing lung damage and disease progression (13).

Following the discovery of the *CFTR* gene and its pathogenic variants, years of research led to the development of targeted therapies known as CFTR modulators. Cystic fibrosis transmembrane conductance regulator modulators are innovative therapies that act directly on the defective CFTR protein to restore its function, addressing the root cause of the disease rather than its consequences. Key CFTR modulators include ivacaftor, the dual combinations lumacaftor-ivacaftor and tezacaftor-ivacaftor, and the triple combinations elexacaftor-tezacaftor-ivacaftor and vanzacaftor-tezacaftor-deutivacaftor (Table 2). The triple combination therapy has been a major breakthrough, showing efficacy in approximately 90% of CF patients with eligible *CFTR* mutations, especially those with the *CFTR* *F508del* variant (NM_000492.4:c.1521_1523delCTT; p.Phe508del). Clinical trials and real-world data have demonstrated significant improvements in lung function, a decrease in pulmonary exacerbations, better nutritional status, and enhanced quality of life. Decreased sweat chloride levels, an important biomarker, indicate restored CFTR function. Moreover, early introduction of therapy may alter the natural course of CF and improve long-term outcomes (14,15).

TABLE 2. Overview of cystic fibrosis transmembrane conductance regulator modulators: mechanism of action, target mutations and European medicines agency - approved age indications

CFTR modulator	Type	Mechanism of action	Targeted mutations	Indicated age for therapy (EU/EMA)*
Ivacaftor (Kalydeco)	Potentiator	Increases CFTR channel opening probability	Gating mutations (e.g. <i>G551D</i>)	> 4 months
Lumacaftor–Ivacaftor (Orkambi)	Corrector + potentiator [†]	Improves CFTR folding and channel gating	Homozygous <i>F508del</i> [‡]	> 2 years
Tezacaftor–Ivacaftor (Symkevi)	Corrector + potentiator	Improves CFTR processing and gating	Homozygous <i>F508del</i> or one responsive mutation	> 6 years
Elexacaftor–Tezacaftor–Ivacaftor (Kaftrio)	Triple therapy	Two correctors plus one potentiator; significantly improves CFTR function	≥ 1 <i>F508del</i> allele	> 2 years
Vanzacaftor–Tezacaftor–Deutivacaftor (in clinical trials)	Triple therapy (next-generation)	Enhanced potency and improved pharmacokinetics	≥ 1 <i>F508del</i> and other mutations (pending approval)	In clinical trials

Commercial brand names are provided in parentheses. *Indicated age refers to the minimum approved age for therapy according to the European medicines agency (EMA). [†]Potentiators increase the open probability of the CFTR channel at the cell surface, whereas correctors improve CFTR protein folding, processing and trafficking to the plasma membrane. [‡]*F508del* refers to the CFTR variant NM_000492.4:c.1521_1523delCTT (p.Phe508del), the most common pathogenic mutation in cystic fibrosis. CFTR - cystic fibrosis transmembrane conductance regulator.

The remarkable improvements in clinical outcomes achieved with CFTR modulators have led some experts to define cystic fibrosis as a “new disease”. Although the prognosis of CF has improved significantly, new challenges have emerged, such as long-term drug safety, high treatment costs, and treatment adherence (16).

Croatia is among European countries with a high prevalence of CFTR *F508del* variant, present in approximately 70–80% of CF alleles, making the majority of Croatian patients eligible for CFTR modulator triple therapy. These modulators have been introduced into clinical practice in Croatia since autumn 2021. According to national data, patients on triple therapy have shown significant improvements in both clinical outcomes and quality of life (17–19). Current national priorities for CF therapy include expanding eligibility for the triple combination of elexacaftor, tezacaftor, and ivacaftor to younger age groups and introducing vanzacaftor/tezacaftor/deutivacaftor into Croatian clinical practice.

Newborn screening in the era of personalised medicine

A novel approach in medicine is focused on the specific needs of each patient and is described by the term “P4 medicine” (predictive, preventive, personalised, participatory). This concept is based on understanding an individual’s genetic features, biomarkers, environmental, and lifestyle factors (20). This knowledge enables clinicians to select the most appropriate preventive or therapeutic approach at the optimal time. At the same time, an increasing number of novel therapeutic options are becoming available for rare diseases, including gene therapies that are already in routine use for certain diseases. However, these therapies are most effective when started early-ideally during the presymptomatic phase of the disease (21).

Newborn screening (NBS) from dried blood spots (DBS) is a diagnostic approach widely recognized as one of the greatest achievements of modern public health, particularly in rare diseases (22). The

fundamental goal of NBS is the early identification of newborns with rare monogenic disorders for which treatment exists and where early intervention reduces morbidity and mortality. For decades, Wilson and Jungner’s principles guided decisions on which diseases to include in NBS programs (23). However, advances in technology and clinical practice have required modifications to these historic criteria (24,25). The number of disorders included in NBS programs differs between countries and may now reach up to 50, depending on local policies (26). In Croatia, NBS was first introduced in 1978 with screening for phenylketonuria, followed by congenital hypothyroidism seven years later. Since 2017, tandem mass spectrometry (MS/MS) has been used in the Croatian NBS program, expanding screening to eight rare diseases. In 2023, molecular technologies were introduced, enabling screening for spinal muscular atrophy (SMA). An overview of the development of NBS in Croatia is presented in Table 3.

Tandem mass spectrometry enables rapid detection of numerous metabolites in DBS and has transformed NBS from single-disease testing into a platform capable of identifying more than 40 conditions simultaneously. Today, MS/MS is widely used as a first-tier test for inborn errors of metabo-

TABLE 3. Milestones in the implementation of newborn screening in Croatia

Year	Test/Condition introduced*	Technology used†
1978	Phenylketonuria	Biochemical test from DBS
1985	Congenital hypothyroidism	Hormone analysis
2017	Eight rare diseases	Tandem mass spectrometry
2023	Spinal muscular atrophy	Molecular methods (PCR)
2023	Expanded Croatian NBS panel	Customized NGS panel (confirmatory testing)

*Refers to the condition(s) newly included in the national newborn screening (NBS) programme in the specified year.
†Technology primarily used for screening or confirmatory testing at the time of implementation. DBS - dried blood spots. NBS - newborn screening. NGS - next-generation sequencing. PCR - polymerase chain reaction.

lism (IEM). Nevertheless, it has well-known limitations, including sensitivity to sample quality, the risk of false-positive and false-negative results, and the inability to detect disorders without measurable metabolite changes in blood (27). The strengths and limitations of different NBS technologies are summarized in Table 4.

TABLE 4. Technologies in newborn screening – advantages, limitations and clinical applications

Technology	Advantages	Limitations*	Application
MS/MS	Rapid multi-metabolite analysis; detects > 40 conditions simultaneously	Sample-quality sensitive; risk of FP/FN results; limited to metabolite-detectable disorders	IEM, first-tier NBS
gNBS (PCR, MLPA, ddPCR)	Direct variant detection; fast and precise	Limited to predefined target genes; requires prior assay validation	SMA, SCID
NGS	Wide gene–disease coverage (~480 genes)	High cost; challenges in VUS interpretation; requires advanced bioinformatics expertise	Confirmatory testing after positive NBS results
Untargeted metabolomics	Discovery of novel biomarkers; deeper phenotypic insight	Lack of standardization; requires extensive clinical validation	Potential future application

*These represent potential limitations of screening technologies, reflecting the possibility of incorrect classification due to analytical constraints, biological variability, or incomplete genomic coverage. ddPCR - droplet digital polymerase chain reaction. FP - false positive. FN - false negative. gNBS - genomic newborn screening. IEM - inborn errors of metabolism. MLPA - multiplex ligation-dependent probe amplification. MS/MS - tandem mass spectrometry. NBS - newborn screening. NGS - next-generation sequencing. PCR - polymerase chain reaction. SCID - severe combined immunodeficiency. SMA - spinal muscular atrophy. VUS - variant of uncertain significance.

The extraction of genetic material from DBS has enabled gNBS, now widely adopted in Europe as a first- or second-tier test for SMA and severe combined immunodeficiency. Methods used include real-time polymerase chain reaction (PCR), digital droplet PCR, and multiplex ligation-dependent probe amplification (28). In parallel, the development of NGS has accelerated progress in genetics. Following positive biochemical screening results, a customized NGS panel is now used as a confirmatory diagnostic test for all disorders included in the Croatian NBS program (29).

In the current era of genomic medicine, NBS faces new challenges. The gNBS could significantly expand the number of screened conditions if used as a first-tier test, while reducing false positives, shortening diagnostic times, improving predictive values, and broadening clinical utility (30). Several international pilot studies are evaluating the feasibility of gNBS panels, which typically include a median of 480 gene-disease associations (31). However, broader screening does not automatically mean a better NBS program. Integration of molecular technologies into NBS offers numerous advantages, but also raises questions such as legal and ethical concerns, interpretation of results, and infrastructural requirements, as summarized in Table 5. There is also a need for effective systems for long-term follow-up and care for persons diagnosed with a genetic condition through NBS (32).

At the same time, untargeted metabolomics using quadrupole time-of-flight (Q-TOF) or tandem Orbitrap instruments is emerging as a complementary approach. This approach offers greater insights into phenotypes of disease and can detect novel and known disease biomarkers. However, current methods still need comprehensive validation and careful selection of clinically significant biomarkers for examination (33).

Today and in the near future, designing an effective NBS demands strategic combination of advanced metabolomics with biochemical methods, and genomic technologies in standardized protocols. Such approaches also require the availability of appropriate therapeutic options for the screened diseases. Current consensus suggests

TABLE 5. Challenges in integrating genomic newborn screening into clinical practice in Croatia

Category	Key challenges
Ethical and legal	Data privacy in Croatia; informed consent procedures; potential psychological impact on families; long-term storage and secondary use of genomic data
Interpretation	Need for additional training of healthcare professionals; standardization of sequencing workflows; quality control; interpretation of VUS; reporting of predisposition genes
Infrastructure	Investment in laboratory and bioinformatics capacity; establishment of multidisciplinary teams; development of long-term clinical follow-up systems
Societal	Public and patient engagement; acceptance of genomic technologies; cost considerations and equity of access

The listed challenges reflect current organizational, regulatory and infrastructural aspects relevant to the implementation of genomic newborn screening within the Croatian national healthcare system, based on available national programme data and implementation experience. VUS - variant of uncertain significance.

that the introduction of genomic methods into NBS should be justified only for disorders with early childhood onset and for diseases with effective treatments that can cure, prevent, or slow disease progression (34,35). The future direction of NBS is closely linked to personalised medicine, integrating genetic findings into individualized treatment and follow-up strategies for each infant. It will require integration of NBS with health information systems, artificial intelligence systems, and longitudinal follow-up of infants identified through NBS to track their long-term health outcomes and treatment effectiveness. The multi-omics approach offers exciting potential to expand the scope of NBS programs in the future (36).

Along with this new potential, sustaining, and advancing NBS programs present an ongoing challenge. Despite all current and future technological possibilities, the benefits of NBS for the child must always outweigh the risks. Any revision of NBS criteria should include medical, legal, ethical, and social considerations. Furthermore, it should engage patients, key stakeholders, and the wider community.

Spinal muscular atrophy in newborn screening - Treatment advantages

Spinal muscular atrophy shows how NBS programmes can improve disease outcomes through early treatment initiation (29,37). It is a severe neuromuscular disorder most often caused in about 95% of cases by homozygous deletion of the *SMN1* gene, and is characterized by high morbidity and mortality, particularly in its most severe form – Type 1 (38,39). The introduction of disease-modifying therapies has revolutionised the therapeutic landscape of SMA, shifting the focus from palliative care to a promising curative strategy when treatment begins before symptom onset (40,41). Implementing NBS for SMA enables early identification of affected infants before symptoms appear, providing a unique opportunity for early medical intervention (29,37). Presymptomatic therapy yields the best outcomes, as motor neurons remain responsive to therapy before irreversible damage occurs (42,43).

Revolutionary therapeutic landscape

Three ground-breaking therapies have transformed outcomes for SMA patients: nusinersen,

onasemnogene abeparvovec, and risdiplam (40,42,43). Each therapy targets the core pathophysiology of SMA. Nusinersen, administered intrathecally, is an antisense oligonucleotide that modulates *SMN2* pre-mRNA splicing to enhance the synthesis of functional SMN protein (40,44). Clinical studies show remarkable efficacy when initiated presymptomatically, with patients achieving motor milestones that sharply contrast with the natural history (40,42). The NURTURE study reported 100% survival without permanent ventilation and near-normal motor development in most participants treated presymptomatically (40,45). Onasemnogene abeparvovec, the first gene therapy for SMA, delivers a functional *SMN1* gene copy via an adeno-associated virus vector; when administered presymptomatically, infants reach milestones typically unattainable in untreated SMA Type 1 (43). Risdiplam is an orally administered small molecule that enhances *SMN2* pre-mRNA splicing and increases production of functional SMN protein. Its oral bioavailability and favourable safety profile support long-term therapy, with best results when started presymptomatically (40,43). The three available disease-modifying therapies are summarised in Table 6.

TABLE 6. Disease-modifying therapies for spinal muscular atrophy

Therapy	Type	Drug administration*	Mechanism of action	Key evidence†
Nusinersen	Antisense oligonucleotide	Intrathecal injection	Modification of <i>SMN2</i> pre-mRNA splicing leading to increased production of functional SMN protein	NURTURE study: presymptomatically treated infants showed survival and attainment of motor milestones not typically observed in untreated SMA type 1
Onasemnogene abeparvovec	Gene therapy	Intravenous infusion (single dose)‡	AAV9-mediated delivery of a functional <i>SMN1</i> gene resulting in increased SMN protein expression	Presymptomatic infants achieved major motor milestones rarely observed in untreated SMA type 1
Risdiplam	Small molecule	Oral administration	Modification of <i>SMN2</i> splicing resulting in increased systemic SMN protein levels	Demonstrated sustained motor function improvement, with greatest benefit when initiated presymptomatically

*Drug administration indicates the route and mode of delivery used in clinical practice. †Key evidence summarizes findings from pivotal clinical trials supporting the efficacy of early or presymptomatic treatment. ‡“Single dose” refers to one-time systemic gene replacement therapy administered as a single intravenous infusion. AAV9 - adeno-associated virus vector 9. SMA - Spinal muscular atrophy. SMA type 1 - most severe early-onset form of SMA characterized by symptom onset in infancy and rapid motor neuron degeneration. SMN - Survival motor neuron.

The advantage of presymptomatic over symptomatic treatment is substantial and consistent across all available therapies (40,42). Natural history studies show that patients with SMA Type 1 rarely achieve independent sitting, with a median survival without ventilatory support of ~10.5 months (38). In contrast, presymptomatically treated patients frequently achieve independent sitting, standing, and even walking (40,42,43). Long-term follow-up confirms durable benefits: treated patients show normal growth, preserved swallowing, fewer respiratory complications, and markedly improved quality of life (43,46,47). While symptomatic patients may respond, they seldom reach the developmental milestones seen with presymptomatic treatment (40,42). The therapeutic window is therefore critical; delays in therapy initiation can compromise outcomes (41-43). A comparison of natural history *versus* presymptomatic outcomes is shown in Table 7.

The Croatian SMA NBS pilot study, conducted at the University Hospital Centre Zagreb, established a clinical pathway from screening-to-treatment (29). During one year of the study, five SMA patients were identified among 32,655 screened newborns. All five patients immediately received specialised neuromuscular evaluation and rapid therapy introduction. The success of the pilot study is reflected both in analytical performance and in the clinical outcomes achieved through ear-

ly therapy initiation. All infants identified in the first year began treatment before symptom onset, with constant monitoring demonstrating preserved motor function and age-appropriate milestones (29). This real-world experience verifies the benefits of presymptomatic treatment observed in clinical trials (40,42,43). The multidisciplinary approach ensures a seamless transition from screening to treatment and serves as an outline for effective clinical implementation (29). The Croatian pilot results are summarised in Table 8.

Furthermore, newborn screening supports a personalised-medicine approach that integrates genetic and clinical factors. Examination of *SMN2* gene copy number leads treatment intensity, as fewer copies generally predict more severe disease type (39,44). Newly developed assessment tools, including motor scales for presymptomatic infants and biomarkers such as compound muscle action potentials, enable accurate monitoring of treatment response and disease progression (40,42).

Economic and societal impact

Patients treated presymptomatically require fewer hospitalisations, fewer respiratory interventions, and less supportive care than those treated after symptom onset (40,46). Preservation of motor function mitigates long-term disability-related costs and extends productive years of life. Family

TABLE 7. Clinical outcomes in spinal muscular atrophy: natural history vs. presymptomatic treatment

Outcome	Natural history (SMA Type 1)	Presymptomatic treatment*
Survival	Median survival 10.5 months without permanent ventilatory support	> 95% survival without need for permanent ventilation
Motor milestones	Independent sitting rarely achieved	Independent sitting, standing and walking frequently achieved when treatment is initiated presymptomatically
Respiratory function	Recurrent infections and early respiratory failure	Preserved respiratory function with fewer complications
Quality of life	Severe motor disability and high caregiver burden	Improved growth and swallowing, reduced disease burden and better family well-being

This comparison summarizes published evidence on the natural history of untreated SMA type 1 and outcomes observed in infants receiving disease-modifying therapy initiated during the presymptomatic stage (46,47). Outcomes are based on clinical trial and observational cohort data demonstrating substantially improved survival and motor development with early treatment. SMA - spinal muscular atrophy.

TABLE 8. Croatian spinal muscular atrophy newborn screening pilot study (2022–2023)

Parameter	Results
Period	1 year
Number of newborns screened	32,655
SMA patients identified	5
Time of diagnosis	Before symptom onset
Intervention	Immediate specialist evaluation plus early therapy
Outcome	Preserved motor function; age-appropriate milestones

This table summarizes the initial real-world outcomes of implementing newborn screening for spinal muscular atrophy, demonstrating early identification of affected infants and timely initiation of therapy prior to symptom onset, which is associated with improved motor development and clinical prognosis. The data reflect results from the national newborn screening programme in Croatia during the first year of SMA screening implementation (29). SMA - spinal muscular atrophy.

quality of life improves substantially with pre-symptomatic therapy, reducing caregiver burden and enhancing family functioning (46).

Addition of SMA to the existing national screening programme in Croatia, together with modern SMA treatments, demonstrates precision medicine in action: early recognition leads to transformative clinical outcomes (40,41,43). The results of the Croatian pilot study confirm that well-organised screening-to-treatment pathways deliver the benefits of presymptomatic intervention in real-world clinical practice (29). The body of evidence supports NBS for SMA as a vital component of comprehensive care, ensuring affected infants receive life-changing therapies at the optimal time (40,42,43).

Hereditary malignant diseases

Hereditary malignant diseases often present as hereditary cancer syndromes (HCSs), groups of cancers caused by inherited, germline pathogenic variants that typically follow recognizable clinical patterns and affect multiple organs. Rare congenital disorders, such as Bloom syndrome, Fanconi

anemia, and ataxia-telangiectasia, show broader multi-organ involvement and are usually inherited in an autosomal-recessive pattern, caused by biallelic mutations in DNA-repair genes. These often present with immunodeficiency and early-onset cancers (48,49). In contrast, most individuals with HCSs appear clinically healthy, except for an increased lifetime cancer risk due to inherited germline pathogenic variants (GPVs) in key genes such as tumour suppressors, oncogenes, or mismatch repair (MMR) genes. These GPVs typically follow autosomal dominant inheritance, with a 50% chance of transmission. Although a single inherited GPV does not directly cause cancer because cellular repair mechanisms remain intact, only a few additional somatic mutations are needed to trigger malignant transformation, leading to earlier cancer onset than in the general population.

Hereditary cancer syndromes account for about 10% of all malignancies, yet many remain undiagnosed. Common indicators include early-onset cancers, multiple primary tumours, family history, and rare presentations like male breast cancer (50–52).

Most HCS-related genes are tumour suppressors (e.g., *BRCA1* – breast cancer 1; *BRCA2* – breast cancer 2; *MLH1* – mutL homolog 1; *MSH2* – mutS homolog 2; *RB1* – RB transcriptional corepressor 1), where cancer typically develops after a “second hit” inactivates the remaining functional allele. A smaller number of HCSs involve inherited activating mutations in oncogenes, such as *RET* (ret proto-oncogene receptor tyrosine kinase) in multiple endocrine neoplasia types 2A and 2B (53). A single gene can predispose to several cancer types, while the same cancer may arise from mutations in different genes (Table 9). For example, GPVs in *MMR* genes cause Lynch syndrome (colorectal, endometrial, ovarian, gastric cancers), while *TP53* (tumour protein p53) and *CHEK2* (checkpoint kinase 2) genes are linked to Li-Fraumeni syndrome (leukaemia, breast cancer, sarcoma, brain tumours). Pathogenic variants in the *BRCA1* and *BRCA2* gene are responsible for hereditary breast and ovarian cancer syndrome, increasing the risk of breast, ovarian, prostate, and pancreatic cancers. Beyond

TABLE 9. Hereditary cancer predisposition syndromes in clinical oncology

Syndrome	Tumour type	Mode of inheritance	Genes
Hereditary breast cancer and ovarian cancer syndrome	Breast cancer Ovarian cancer Prostate cancer Pancreatic cancer	Dominant	<i>BRCA1</i> <i>BRCA2</i>
	Fanconi anemia/medulloblastoma	Recessive	<i>BRCA2</i>
HNPCC, including “Lynch II” syndrome	Colon cancer Endometrial cancer Ovarian cancer Renal pelvis cancers Ureteral cancers Pancreatic cancer Stomach and small bowel cancers Hepatobiliary cancers	Dominant	<i>MLH1</i> <i>MSH2</i> <i>MSH6</i>
	Soft tissue sarcoma Breast cancer Osteosarcoma Leukaemia, Brain tumours Adrenocortical carcinoma	Dominant	<i>TP53</i> <i>CHEK2</i>
Cowden Syndrome	Breast cancer Thyroid cancer Endometrial and other cancers	Dominant	<i>PTEN</i>
FAP, AFAP	Colon cancer	Dominant	<i>APC</i>
MAP	Colon cancer	Recessive	<i>MUTYH</i>
Hereditary gastric cancer	Stomach cancers	Dominant	<i>CDH1</i>
Juvenile polyposis	Gastrointestinal cancers Pancreatic cancer	Dominant	<i>SMAD4</i> <i>BMPR1A</i>
Peutz-Jeghers syndrome	Colon cancer Small bowel cancer Breast cancer Ovarian cancer Pancreatic cancer	Dominant	<i>STK11</i>
Familial gastrointestinal stromal tumour	Gastrointestinal stromal tumours	Dominant	<i>KIT</i>
Hereditary melanoma pancreatic cancer syndrome	Pancreatic cancer Melanoma	Dominant	<i>CDKN2A</i>
Bloom syndrome	Leukaemia Carcinoma of the tongue Squamous cancers Wilms’ tumour Colon cancer	Recessive	<i>BLM</i>
	Lymphoma Glioma Medulloblastoma Rhabdomyosarcoma	Recessive	<i>NBS1</i>
Retinoblastoma	Retinoblastoma Osteosarcoma	Dominant	<i>RB1</i>
Hereditary paraganglioma	Paraganglioma Pheochromocytoma	Dominant	<i>SDHD</i> <i>SDHC</i> <i>SDHB</i>

This table provides a selective overview of clinically relevant hereditary cancer predisposition syndromes in oncology and is not intended to represent an exhaustive list of all known syndromes.

AFAP - attenuated familial adenomatous polyposis. APC - adenomatous polyposis coli. BLM - Bloom syndrome RecQ like helicase. BMPRI1A - bone morphogenetic protein receptor type 1A. BRCA1 - breast cancer 1. BRCA2 - breast cancer 2. CDH1 - cadherin 1. CDKN2A - cyclin-dependent kinase inhibitor 2A. CHEK2 - checkpoint kinase 2. FAP - familial adenomatous polyposis. HNPCC - hereditary nonpolyposis colorectal cancer. KIT - KIT proto-oncogene receptor tyrosine kinase. MAP - MUTYH-associated polyposis. MLH1 - mutL homolog 1. MSH2 - mutS homolog 2. MSH6 - mutS homolog 6. MUTYH - mutY DNA glycosylase. NBN (NBS1) - nibrin. PTEN - phosphatase and tensin homolog. RB1 - RB transcriptional corepressor 1. SDHB - succinate dehydrogenase complex iron sulfur subunit B. SDHC - succinate dehydrogenase complex subunit C. SDHD - succinate dehydrogenase complex subunit D. SMAD4 - SMAD family member 4. STK11 - serine/threonine kinase 11. TP53 - tumour protein p53.

BRCA1 and *BRCA2*, variants in genes like *ATM* (ataxia-telangiectasia mutated), *PALB2* (partner and localizer of *BRCA2*), *BRIP1* (*BRCA1* interacting helicase 1), *STK11* (serine/threonine kinase 11), and *NBN* (nibrin) also contribute to a moderate hereditary predisposition for breast cancer (51,52).

It is important to emphasize that individuals do not inherit cancer itself but rather GPs in specific genes that increase susceptibility to certain tumour types. These variants are classified according to their penetrance into high, moderate and low-risk categories. High-risk (highly penetrant) genes, such as *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, *APC* and *TP53*, are linked with HCSs such as HBOC, Lynch syndromes, FAP and Li-Fraumeni syndrome. Individuals with GPs in these genes may have a 5 to 50-fold increased risk compared to the general population. Moderate-risk genes, including *ATM*, *BRIP1*, *CHEK2* and *PALB2*, give a 1.5 to 5-fold increased cancer risk. Low-risk variants, while individually associated with only a slight increase in risk (up to 1.5-fold), are common in the population, and their cumulative effect may be clinically relevant (54).

The molecular basis of hereditary malignant diseases lies in GPs within the genome that affect gene function or expression. The American college of medical genetics (ACMG) has established a five-tier system classifying genetic variants: pathogenic (P), likely pathogenic (LP), of uncertain significance (VUS), likely benign (LB), and benign (B) (55). In oncology, the International Agency for research on cancer (IARC) further refines this by applying quantitative probability thresholds to assess dis-

ease causality (Table 10) (56). These classification schemes lead the clinical interpretations of genetic results. Gene variants graded as P or LP are counted as clinically actionable, they give information about treatment and disease-specific surveillance. However, VUSs are still a key challenge due to their doubtful impact on gene function and risk of disease. Continuous follow-up and genetic counselling are therefore essential for such patients so determination of VUS clinical relevance over time can be done. The cancer development has a multifactorial basis, individuals with suspected HCSs are recommended to take genetic testing (NGS and multi-gene panels focused on cancer susceptibility genes). Compared to traditional methods, NGS is faster, broader, and more cost-effective. It can be used for single-genes, targeted panels, whole exomes, or even whole genomes, giving complete genetic information but keeping controllable costs (57,58). In oncology, multi-gene panel NGS testing is favoured due to its higher analytical sensitivity and specificity. By focusing on disease-relevant genes, it simplifies interpretation and improves diagnostic accuracy. Genetic testing identifies GPs that increase cancer risk and has several key clinical applications (59). First, certain variants predict treatment response, for example, *BRCA1* and *BRCA2* gene variants indicate potential benefit from PARP inhibitors, while *MMR* gene variants can guide immunotherapy decisions (60,61). Second, confirming a HCS at the molecular level enables tailored preventive strategies, including enhanced surveillance or prophylactic surgery, which can significantly improve outcomes (62,63).

TABLE 10. Classification system of genetic variants according to the International agency for research on cancer

Class*	Description [†]	Probability of being pathogenic	Counseling consequences [‡]
1	Benign	< 0.001	Considered equivalent to “no pathogenic variant detected”; routine population-based screening recommended
2	Likely benign	0.010-0.049	Managed as negative test result; standard clinical surveillance based on general risk
3	VUS	0.050-0.949	Clinical management guided by personal and family history; periodic variant re-evaluation
4	Likely pathogenic	0.950-0.990	Genetic counselling and intensified surveillance according to high-risk management guidelines
5	Pathogenic	> 0.990	Full high-risk management including tailored surveillance, preventive strategies and cascade testing of relatives

*Classes refer to the IARC five-tier classification of germline genetic variants in cancer susceptibility genes (56). [†]Description indicates the clinical interpretation of the variant classification. [‡]Counselling consequences refer to recommendations for genetic counselling, clinical surveillance and risk management of the proband and at-risk family members. IARC - International agency for research on cancer. VUS - variant of uncertain significance.

Third, identifying a GPV in a patient allows for cascade testing in family members, facilitating individualized preventive or therapeutic plans (64,65).

All genetic testing candidates must take pre-test counselling and give informed consent. Counselling should cover the purpose and limitations of the genetic test, likely outcomes, implications for family members and privacy concerns.

The discovery of cancer-predisposing genes and improved diagnosis of hereditary malignancies represent significant progress in translational medicine today, in the current era of personalised medicine. Future investigations should spotlight strategies for tumour development prevention and progression prevention in germline pathogenic variants carriers.

From genetics to treatment: a personalised approach to oncology

Precision oncology moves beyond the one-size-fits-all model of cancer care. It emphasises therapies customized to the patient's genetic and molecular features and directed toward cancer-specific mutations (66,67). This approach is driven by the recognition that the same mutations can occur in

tumours from different organs, allowing treatment efficacy to be independent of tumour origin. The goal is to improve outcomes by using treatments tailored to each patient's unique genetic changes.

Cancer is a disease of cellular evolution. Over time, cancer cells acquire mutations that confer a survival advantage, enabling uncontrolled growth and resistance to cell death. This reflects a loss of control over the cell cycle, which is regulated by two major gene classes: proto-oncogenes and tumour-suppressor genes. Proto-oncogenes promote normal cell growth, in the case of the mutation, they become oncogenes, with a “gain of function” which leads to uncontrolled cell proliferation. A single mutated allele can be enough to alter gene function. These mutations are typically acquired. Tumour suppressor genes suppress cell growth and programmed cell death. Mutations in these types of genes result in a “loss of function” and commonly require both mutated alleles to be deactivated, often through loss of heterozygosity (LOH). A subclass, DNA repair genes, are tumour suppressors since their loss of function increases the overall mutation rate in other genes. The variations between proto-oncogenes, tumour suppressor, and DNA repair genes are summarised in Table 11.

TABLE 11. Gene categories in cancer

Gene type	Normal role	Effect of mutation	Example genes	Inheritance pattern
Proto-oncogenes	Promote growth/division	Gain of function → oncogene activation, and uncontrolled proliferation	<i>KRAS, BRAF, EGFR</i>	Somatic (predominantly)
Tumour suppressor genes	Inhibit growth, trigger apoptosis	Loss of function → uncontrolled cell growth	<i>TP53, RB1, APC</i>	Germline with somatic "second hit" or somatic only
DNA repair genes	Maintain genomic stability and DNA integrity	Loss of function → accumulation of mutations	<i>BRCA1, BRCA2, MSH2, MLH1</i>	Germline or somatic

APC - adenomatous polyposis coli. *BRAF* - B-Raf proto-oncogene, serine/threonine kinase. *BRCA1* - breast cancer 1. *BRCA2* - breast cancer 2. *EGFR* - epidermal growth factor receptor. *KRAS* - Kirsten rat sarcoma viral oncogene homolog. *MLH1* - mutL homolog 1. *MSH2* - mutS homolog 2. *RB1* - RB transcriptional corepressor 1. *TP53* - tumour protein p53.

Key mutations, critical for a tumour's growth and progression, are referred to as driver mutations. They are often point mutations and are rarely lost due to weak negative selection (68). Tumours typically have about four driver mutations, though this varies by cancer type (69). Driver mutations are characterized by their recurrence across many patients and at specific "hotspots" (70). Most other mutations are passenger mutations, which are not critical for tumour growth, but recent research suggests they can have a cumulative effect on the tumour's characteristics (71).

Most tumour mutations are somatic (acquired) due to environmental factors or aging; only 5-10% are germline (inherited) (72). Unlike germline mutations, which are in every cell, somatic mutations are only in the cells where they occurred, leading to clonal heterogeneity. As a tumour grows, different subpopulations of cells, or subclones, develop unique mutations (73).

Cancer treatments act as a powerful selective force (74). A drug may kill the dominant, "naive" cancer cells, but it selects for and promotes the growth of a small fraction of cells that have acquired resistance mutations. This resistant subclone then becomes the dominant population, explaining why subsequent therapies are often less effective.

Mutations can be detected using tissue biopsy or a non-invasive liquid biopsy, which identifies tumour-derived DNA fragments circulating in the

bloodstream (75). Liquid biopsies can detect mutations from multiple subclones.

Today, NGS is the primary method for analysing cancer genomics (76). It can detect various mutations, for example, single nucleotide polymorphisms (SNPs), small deletions and insertions (indels), and larger changes like copy number variations (CNVs). The variant allele frequency (VAF) helps differentiate between germline and somatic mutations; a high VAF (50-100%) suggests a germline mutation, while somatic mutations can have a very low VAF (77).

Somatic mutations leave characteristic patterns called mutational signatures, with more than 80 identified by COSMIC (78). These signatures fall into three types:

- Type 1: caused by exogenous or endogenous processes (e.g., UV radiation).
- Type 2: linked to defective DNA repair mechanisms (e.g., *BRCA1/BRCA2* mutations).
- Type 3: caused by unknown processes.

The classification of mutational signatures and their therapeutic implications is presented in Table 12.

Understanding these signatures is clinically valuable as a predictor of therapeutic response. For example, tumours with a specific signature indicating a defect in homologous recombination are sensitive to platinum-based therapies or PARP inhibitors. A high tumour mutational burden (TMB)

TABLE 12. Mutational signatures and their therapy implications

Signature type	Main cause	Example	Therapy relevance
Type 1	Exogenous and endogenous mutational processes	UV-induced mutations	May predict response to immunotherapy in melanoma
Type 2	DNA repair defects	<i>BRCA1/2</i> deficiency	Increased sensitivity to platinum-based chemotherapy and PARP inhibitors
Type 3	Unknown mechanisms	–	Currently under research

Signature types represent simplified categories of mutational processes associated with specific biological mechanisms and therapeutic vulnerabilities. *BRCA1* - breast cancer 1. *BRCA2* - breast cancer 2. PARP - poly(ADP-ribose) polymerase. UV - ultraviolet.

signature or a defect in the mismatch repair (MMR) system that leads to high microsatellite instability (MSI-H) are both excellent predictors of response to immunotherapy, regardless of the tumour’s location (79).

Different to conventional chemotherapy, precision oncology drugs have high specificity for molecular targets (80). These drugs fall into two main categories: small-molecule drugs and biologics. Chemically synthesized small-molecule drugs act on intracellular or extracellular targets (*e.g.*, kinase inhibitors), while biologic drugs, typically antibodies, target extracellular targets (*e.g.*, cell receptor inhibitors). Although precision oncology has potential, it faces challenges such as tumour hetero-

geneity, considerable healthcare costs, and limited access to genetic testing (81). Consequently, to address these concerns, innovative clinical trial designs have been developed to better evaluate targeted therapies and enable their implementation in precision oncology (82,83). Table 13 summarizes contemporary adaptive clinical trial designs used in precision oncology to match targeted therapies with specific genomic alterations either across or within tumour types.

All multifaceted decisions are often reached by a multidisciplinary team of experts.

Results of molecular profiling precisely direct the selection of targeted drugs that work on specific molecular pathways. Key patterns include:

TABLE 13. Innovative clinical trial designs in precision oncology

Trial design	Inclusion criteria	Purpose	Example
N-of-1*	Single patient with a defined molecular alteration	Evaluation of individualized targeted therapy in a single patient	Rare tumours with unique actionable mutations
Basket	Different tumour types sharing the same molecular alteration	Assessment of efficacy of mutation-targeted therapy across cancer types	NTRK inhibitor trials
Umbrella	Single tumour type with different molecular subgroups	Allocation of targeted therapies based on molecular profiling	Lung cancer umbrella trials
Super-umbrella	Hybrid design combining basket and umbrella approaches	Large adaptive master protocol evaluating multiple targets and therapies	Master protocols in precision oncology

This table summarizes contemporary adaptive clinical trial designs used in precision oncology to match targeted therapies with specific genomic alterations across or within tumour types. *N-of-1 trial refers to a single-patient clinical study in which treatment is selected based on the individual molecular profile, allowing direct assessment of therapeutic benefit in that specific patient. NTRK - Neurotrophic tyrosine receptor kinase.

- Kinase and receptor mutations: tumours with *BRAF*, *EGFR*, and *ALK* gene mutations exhibit constant growth signals and can be treated with kinase inhibitors. Amplification of the *ERBB2* (*HER2*) gene is targeted with monoclonal antibodies (trastuzumab).
- DNA repair mutations: tumours with *BRCA1/BRCA2* mutations have damaged DNA repair, and consequently are sensitive to PARP inhibitors. Tumours with a defect in the *MMR* system respond well to immunotherapy.
- Other targets: mutations in genes such as *IDH1*, *PIK3CA*, *KRAS G12C*, *NTRK*, and *RET* can be treated with specific inhibitors.

Detailed molecular profiling, from driver mutations and mutational signatures to understanding tumour heterogeneity, is the foundation of personalised cancer treatment. As our knowledge of tumour genetics progresses, new targeted treatments continue to emerge. These advances have turned precision oncology into standard practice, allowing clinicians to tailor therapy decisions according to the patient's unique genetic characteristics.

Pharmacogenetics and individualisation of therapy

The concept of individualising drug therapy based on genetic information is one of the key implementations of personalised medicine today. Pharmacogenetics, the study of how genetic variation affects individual responses to therapy, aims to optimise drugs selection and minimise adverse drug reactions (ADRs). As early as the mid-20th century, discoveries of inherited enzyme insufficiencies, for example, glucose-6-phosphate dehydrogenase (G6PD) deficiency and N-acetyltransferase polymorphisms revealed that genetics significantly modulates drug metabolism and toxicity (84,85). Today, with the advent of NGS and bioinformatics, pharmacogenetics has expanded from single gene-drug interactions to pharmacogenomics, genome-wide approaches that consider the complex interplay of multiple genes and pathways (86,87). Currently, pharmacogenomics is clinically relevant for a growing number of medica-

tions, with actionable pharmacogenes guiding drug selection and dosing to improve efficacy and reduce ADRs. Implementation is supported by expert societies such as the Dutch pharmacogenetics working group (DPWG) and the clinical pharmacogenetics implementation consortium (CPIC), which have published evidence-based guidelines for more than 160 gene-drug pairs (88-91). Integrating pharmacogenetic data into clinical workflows has been associated with improved safety, efficacy, and greater cost-effectiveness, particularly for medicines with narrow therapeutic index or high ADR risk (92).

At the core of pharmacogenomics are inherited variants, especially in genes encoding drug-metabolising enzymes, transporters, receptors, drug targets, and immune modulators, that influence how individuals process and respond to medications throughout their lives (86,87,93). Therefore, pharmacogenomic testing focuses on germline sequence variants that influence individual variability in drug response, covering pharmacokinetics (drug metabolism) and pharmacodynamics (drug targets and sensitivity) (94). It is estimated that variations in genes involved in pharmacokinetics and pharmacodynamics explain about 20-30% of variability in drug response (92). Unlike inherited disease genetics, in pharmacogenetics germline sequence variants are not classified by the traditional pathogenicity categories used in medical genetics, because they primarily describe the impact on drug response and not disease causation. Major pharmacogenetics resources, such as PharmGKB (Pharmacogenomics knowledgebase), and newly established ClinPGx (Clinical pharmacogenomics), as well as CPIC and DPWG, classify gene-drug interactions by systematically evaluating drug-gene pair clinical relevance and actionability for drug prescribing decisions (88,95,96). These resources provide evidence-based recommendations regarding drug selection, dosage adjustment, or consideration of alternative therapies based on genotype-predicted phenotypes such as poor, intermediate, normal, or ultra-rapid metabolizer status, as well as enzyme activity scores and drug transporter functionality.

Genetic polymorphisms of cytochrome P450 (CYP) enzymes significantly influence individual responses to drugs, susceptibility to toxicity, and cancer risk. They are crucial for metabolising endogenous substances, xenobiotics, and carcinogens. Variants in CYP genes may lead to loss of enzyme function, reduced expression, altered substrate specificity, or increased enzyme activity (97,98). A key step in pharmacogenomics is translating genotype into phenotype, enabling functional predictions that guide drug therapy. For drug-metabolising enzymes such as CYPs, allele combinations define metabolic phenotypes of individuals, ranging from three to five categories depending on the specific enzyme and functional allele composition. These phenotypes include poor metabolizers (PMs; two nonfunctional alleles); intermediate metabolizers (IMs; one normal function and one nonfunctional allele, or two decreased function alleles), normal metabolizers (NMs; two normal function alleles, or one normal function and one decreased function allele), rapid metabolizers (RMs; one normal function and one increased function allele), and ultrarapid metabolizers (UMs; multiple active gene copies or two increased function alleles) (94,99). A similar genotype-to-phenotype translation applies to drug transporters (ABCG2, SLCO1B1), where variants alter substrate transport rather than metabolism. Functional categories range from poor, decreased, normal, to increased function phenotypes, influencing drug exposure and toxicity risk (99,100).

The most relevant pharmacogenomic markers are those with high-level evidence for clinical actionability by CPIC and DPWG, and those recognised by regulatory agencies such as the European medicines agency (EMA) and the United States Food and drug administration (FDA). The key pharmacogenomic genes include *ABCG2*, *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *HLA-B*, *NUDT15*, *SLCO1B1*, *TPMT*, *UGT1A1*, *VKORC1* and others (85,86,88,89,91,95). These genes are involved in the metabolism of numerous drugs, with common clinical applications across multiple therapeutic areas: psychiatry (selective serotonin reuptake inhibitors (SSRIs): *CYP2B6*, *CYP2C19*, *CYP2D6*); cardiology (clopidogrel: *CYP2C19*; mavacamten: *CYP2C19*;

statins: *ABCG2*, *SLCO1B1*; warfarin: *CYP2C9*, *VKORC1*); oncology (fluoropyrimidines: *DPYD*; tamoxifen: *CYP2D6*; irinotecan: *UGT1A1*); pain management (opioids: *CYP2D6*); infectious diseases (HLA-B57:01 predicts abacavir hypersensitivity); immunosuppressive therapy (tacrolimus: *CYP3A5*; azathioprine: *NUDT15*, *TPMT*); and neurology (carbamazepine: *HLA-B15:02*; siponimod: *CYP2C9*), among others. Given the wide range of gene-drug interactions, transitioning from single-gene tests to pre-emptive pharmacogenomic panels is a logical next step. Recent large trials support this approach. The PREPARE study demonstrated that implementing a 12-gene pharmacogenetic panel significantly reduces ADRs across diverse European healthcare systems (89,91). This aligns with recent finding that 98.8% of individuals carry at least one actionable pharmacogenetic variant with therapeutic implications, and 23.3% have at least one specific gene-drug pair for which a therapy adjustment is recommended (101). This is particularly important for older adults and patients with polypharmacy, where pharmacogenetic testing can help to safely manage complex medication regimens and minimise ADRs in these vulnerable populations (102).

Pharmacogenetic information is increasingly incorporated into drug labels and clinical decision support tools, with more than 300 drug-gene pairs recognised by regulatory agencies FDA and EMA (103). Discrepancies exist between agencies in the level and specificity of pharmacogenetic recommendations, but harmonisation efforts are ongoing (92,94). The consensus of actionable pharmacogenomic labelling between the FDA and the EMA is about 50%, and guidelines provided by CPIC and DPWG are only partly implemented into the summary of product characteristics (SmPCs) (104). Pharmacogenomic actionable labelling is the most common classification, and *CYP2D6* most frequent gene in FDA pharmacogenomic labelling (105).

Both the efficacy and the safety of drug therapy vary among populations due to ethnogeographic differences in pharmacogenetic variants. These differences have direct implications for public health, supporting population-specific genotyping strategies and cost-effectiveness models for pharmacogenomic testing. However, there are sig-

nificant inequities in global pharmacogenomic research coverage, with African and some Asian populations being underrepresented, despite their high genetic diversity and unique variant profiles (92,106). Recognising and integrating these differences is crucial for pharmacogenomic implementation in diverse patient groups (86).

While challenges remain in the implementation of pharmacogenomics into health-care systems – a knowledge gap in the health-care workforce, variability in reimbursement, integration with clinical decision systems, interpretation of rare variants – progress is steadily advancing (90,92). Beyond clinical use, pharmacogenomics also informs drug discovery and development (86). Expanding beyond single-gene testing to comprehensive, population-tailored strategies, supported by clinical guidelines, regulatory frameworks, and international collaboration, is essential for personalised medicine advancement.

Antidepressant treatment in breast cancer patients using tamoxifen

Depression is a common and serious concern among breast cancer patients, affecting roughly one-third of women worldwide (107). Not only can depression arise from the psychological burden of having a life-altering and life-threatening illness, but the biological effects of cancer and its treatment can also cause depression. Among women in Croatia, breast cancer is the most frequently diagnosed cancer according to the Croatian Institute for Public Health. Almost 80% of patients with breast cancer have hormone receptor-positive disease, and in these patients, anti-oestrogen therapies are indicated as adjuvant treatment (108).

Tamoxifen, a weak anti-oestrogen prodrug, is metabolized into active metabolites, including 4-hydroxy-tamoxifen, N-desmethyltamoxifen, and 4-hydroxy-N-desmethyl-tamoxifen (endoxifen). The response to tamoxifen may vary among patients and depends partly on the *CYP2D6* genotype (109,110). The *CYP2D6* gene is highly polymorphic and is classified into four metabolic phenotypes: PM, IM, NM, and UM (111,112). The consequences of impaired tamoxifen metabolism are

particularly significant in patients carrying decreased-function alleles (*CYP2D6* *9, *10, *41) or no-function alleles (*3, *4, *5, *6). The distribution of these variants differs across populations (113,114). Patients categorised as PM with inactivating alleles tend to have inferior survival *versus* carriers of the wild-type allele (115). Nevertheless, recent studies show inconsistent results regarding associations between *CYP2D6* alleles and clinical outcomes (116,117).

Individuals with low *CYP2D6* enzyme activity, either due to genetic variants or concomitant usage of strong *CYP2D6* inhibitors, show significantly decreased endoxifen concentrations during tamoxifen therapy (118,119). Some antidepressants, such as SSRIs and serotonin and norepinephrine reuptake inhibitors (SNRIs), are substrates of *CYP2D6* but also inhibit *CYP2D6*, thereby reducing the creation of active tamoxifen metabolites and potentially its anticancer effect. Table 14 summarizes the inhibitory potential of different antidepressants on *CYP2D6*. Inhibitors can convert normal or ultrarapid metabolizers into intermediate or poor metabolizers. This is known as phenoconversion (120). Phenoconversion arises from drug-drug interactions, and when combined with the genetic background, it is referred to as drug-drug-gene interactions. Given its importance, the CPIC guidelines recommend considering *CYP2D6* inhibitors when calculating the activity score (AS) for the *CYP2D6* enzyme (121). Potent *CYP2D6* inhibitors such as paroxetine and fluoxetine substantially reduce endoxifen concentrations in plasma (122). Weaker inhibitors like sertraline and citalopram may also reduce concentrations, but to a lesser extent (123). Based on this, the application of potent inhibitors should be avoided in patients with tamoxifen therapy (124). Nevertheless, the impact of weak-to-moderate inhibitors remains less clear, and interactions with other CYP enzymes involved in tamoxifen metabolism should also be considered (125).

There has been much debate about whether antidepressant use affects breast cancer recurrence. Current evidence does not support the hypothesis that antidepressants worsen prognosis, although some data suggest a potential increased risk that requires further research (126).

TABLE 14. Antidepressants and their inhibitory effect on cytochrome P450 2D6

Antidepressant	CYP2D6 inhibition strength [*]	Effect on endoxifen concentration [†]	Clinical recommendation
Paroxetine, fluoxetine, bupropion	Strong	Markedly reduced	Avoid concomitant use with tamoxifen
Sertraline, citalopram, duloxetine	Moderate to weak	Slightly reduced	Use with caution and monitor clinical response
Venlafaxine, vortioxetine, escitalopram	Minimal or none	No relevant effect	Considered safer alternatives with tamoxifen

^{*}Inhibition strength refers to the degree of cytochrome P450 2D6 enzyme inhibition, categorized according to *in vivo* effects on CYP2D6 metabolic activity and drug–drug interaction potential. [†]Endoxifen is the main active metabolite of tamoxifen formed predominantly *via* CYP2D6-mediated metabolism; reduced CYP2D6 activity may lead to lower endoxifen plasma concentrations and potentially decreased therapeutic efficacy of tamoxifen. CYP2D6 - cytochrome P450 2D6.

According to CPIC and the DPWG, pharmacogenomic testing for *CYP2D6* is recommended to guide tamoxifen dosing, as well as to inform antidepressant prescribing (121,127). Considering the relevance of phenoconversion, CPIC guidelines also recommend including the effect of CYP2D6 inhibitors in activity score calculation. A recently developed CYP2D6 phenoconversion calculator allows manual prediction of CYP2D6 phenotype by integrating genotype, allele count, and concomitant medications, and is summarized in Table 15 (128). In conclusion, depression is a common condition among women with breast cancer receiving tamoxifen and should not be ignored. A conservative management approach emphasizes thoughtful choice of antidepressants to minimize the risk of clinically significant interactions, especially strong CYP2D6 inhibitors (paroxetine, fluoxetine, bupropion). Weak inhibitors, such as venlafaxine and vortioxetine, are generally safe to use with tamoxifen. Pharmacogenomics has an increasingly important role in optimizing therapy and improving outcomes in these patients.

Conclusion

Altogether, these advances confirm the position of laboratory diagnostics as the cornerstone of personalised medicine-bridging genetic discovery with clinical translation and transforming modern healthcare into a predictive, preventive, and truly patient-centred discipline.

TABLE 15. Cytochrome P450 2D6 phenoconversion calculator – Inputs and outputs

Input required	Description	Output
Genotype	Number and functional status of <i>CYP2D6</i> alleles	Base AS
Concomitant medications	List of co-administered <i>CYP2D6</i> inhibitors	Adjusted AS
Final predicted phenotype	PM, IM, NM, UM (after considering inhibitors)	Guides dosing of tamoxifen and antidepressants dosing

Activity score (AS) represents a quantitative measure of CYP2D6 enzymatic function derived from the sum of allele activity values (e.g. 0 for no function, 0.5 for decreased function, 1 for normal function) (128). The adjusted AS accounts for phenoconversion due to concomitant CYP2D6 inhibitors, resulting in a modified predicted metabolizer phenotype. CYP2D6 - cytochrome P450 2D6. IM - intermediate metaboliser. NM - normal metaboliser. PM - poor metaboliser. UM - ultrarapid metaboliser.

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Potential conflict of interest

None declared.

Data availability statement

Not applicable.

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