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Review

Precision Oncology in Practice: Translating Molecular Profiling into Clinical Decisions and Patient Outcomes

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Abstract

Precision oncology has revolutionized the treatment of cancer through changing it from organ-based to using molecular profiling technology with associated biomarkers. In this review, we discuss the uses and applications of molecular profiling technologies such as next-generation sequencing (NGS), liquid biopsies, and multi-omics in the process of profiling cancer tumors and their associated molecular changes. This review aims to present the use of molecular profiling technologies in precision cancer care by enabling accurate diagnosis, biomarker discovery, target therapy selection, and resistance and progression monitoring of cancer therapies. We also discuss the limitations in the application of these technologies in clinical practice due to problems such as proper analysis of the data, standardization, availability, and cost. Molecular tumor boards and knowledge banks will be covered under molecular profiling technology in facilitating clinical decisions. The new developments in molecular technologies, which include those targeting single cell and spatial omics, along with the employment of AI-based analyses and adaptive trials, are described as useful instruments for improving stratification and treatment personalization. Lastly, the review describes the key obstacles that need to be overcome in order to allow wider application of precision oncology in global oncology practice.

1. Introduction: The Paradigm Shift to Personalized Cancer Care

1.1 From Organ-Based to Biomarker-Oriented Oncology: A Historical Overview

For decades, however, the basis of clinical oncology practice was grounded in a paradigm that was based on organ-based and histopathologic principles. Primary consideration was given to the organ from which a tumor arises and its microscopic appearance when establishing the diagnosis, making prognostic evaluations, and selecting treatment options. Even though this paradigm was indeed valuable in many ways, the underlying reality was that it was largely concealing the real biological differences between tumors, which resulted in ineffective patient outcomes. The shift to biomarker-guided care started with breakthrough studies that associated certain molecular lesions with patient phenotypes and therapeutic susceptibilities [1]. The tamoxifen discovery in the case of estrogen receptor (ER)-positive breast cancer and, above all, imatinib in the case of BCR-ABL1^{+} Chronic Myeloid Leukemia (CML) gave unquestionably irrefutable proof-of-concept that a clear target of a defining molecular driver can be used to achieve transformational efficacy. The following milestones triggered a transition to reduce empirical chemotherapy to a rationale-based one. With the subsequent development of high-throughput genomic technologies, which have resulted in next-generation sequencing (NGS), the full-scale molecular annotation of tumors has now become clinically real, and histology has been officially disregarded as the sole determinant of therapy, and a new era in which the molecular nature of the disease defines itself has begun [2] (Figure 1).

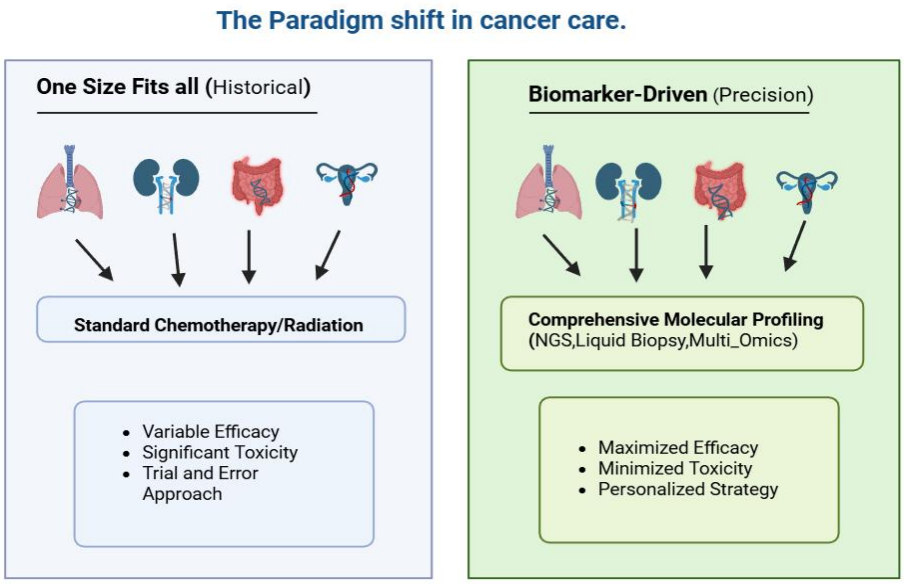

Figure 1. The evolution of oncology: From organ-centric to precision medicine.

1.2 Defining Precision Oncology: Core Principles and Promises

Formally, precision oncology is a therapeutic paradigm in which the treatment of the cancer of a patient is informed by the molecular changes that are observed in his or her tumour, where the goal is to achieve maximum effect and minimal toxicity. Its main tenets are based on a number of pillars: (1) the full molecular characterization of tumor of a patient to find actionable alterations; (2) the correspondence of these alterations to approved or experimental therapeutic options; (3) the active monitoring of disease progression and therapeutic response, typically by liquid biopsy; and (4) the translations of the given molecular information in multipurpose clinical decision-making [3]. The general ideology is to go beyond population averages in standard treatment regimens and provide tailor-made treatment for each patient. This approach will lead to an improved survival rate, improved quality of life due to reduced side effects, and greater utilization of healthcare resources through non-wasteful treatment. The recent advances are centered on the development of tumor-agnostic approvals based on biomarker-based treatment such as Neurotrophic Tyrosine Receptor Kinase (NTRK) gene fusions and High Tumor Mutational Burden (TMB) [4].

1.3 The Engine of Personalization in Molecular Profiling: Beyond the Single-Gene Analysis

Though single-gene tests paved the way in precision oncology, the current cancer treatment landscape is characterized by extensive molecular profiling, especially NGS-based panels. This represents a dramatic move away from a single test, single hypothesis paradigm of testing to a platform of comprehensive information discovery. Apart from somatic mutations, contemporary clinical profiling detects copy number alterations, gene fusions, and even emerging markers such as TMB and microsatellite instability (MSI). Precision oncology is no longer limited to genomics but has expanded to become a multi-omic personalization engine incorporating genomic, transcriptional (e.g., gene expression signatures) and even epigenetic information. Moreover, molecular profiling has been transformed by the emergence of circulating tumor DNA (ctDNA) technology that has allowed the development of a novel method of molecular profiling referred to as liquid biopsy and which can be used for diagnosis, monitoring clonal evolution, and detection of Minimal Residual Disease (MRD) [5].

1.4 Rationale, Purpose and Scope of the Review: Filling the Gap Between Information and Action

Regardless of the rapid evolution of technology, there remains an enormous translational gap from the generation of complicated molecular knowledge to its actual application in practice based on scientific evidence. There are many challenges, such as the interpretation of variants of uncertain significance (VUS), conflicting levels of evidence to act on, tumor heterogeneity, and incorporation of profiling into simplified clinical practice. Our interest in conducting this review is, hence, dictated by the imperative need to create a synergistic understanding of existing knowledge and clinical models that transform molecular profiles into clinical choices [6]. Its main objectives comprise three-fold: the first one to subjectively assess the medical usefulness and shortcomings of the existing and new profiling technologies; the second to map out the institutional, economic, and ethical aspects of the barriers to equity in implementation; and the third to explore the systemic, financial, and ethical challenges of equitable implementation. The scope includes all the spectrum of methods of assay selection and bioinformatic analysis to final treatment recommendation and patient communication, and puts a special emphasis on evidence-based practice [7].

1.5 Article Roadmap

With the purpose of the goals, this review is organized in the following way. Section 2 is a description of the molecular toolbox, including the technical principles, uses, and relative strengths of genomic, transcriptomic, epigenomic, proteomic, and liquid biopsy systems. Section 3 examines the clinical application of this information, including its application in diagnosis, precision therapy and immunomodulation, and addressing therapeutic resistance. Section 4 goes deeper into the important implementation process, examining the clinical interpretation process, the role of MTBs, and logistics considerations of the integration of precision medicine in regular care. Section 5 entrusts the challenge of the limitations to the field by biology, technology, and socioeconomics that persists. Section 6 of the article provides an overview of the future of the field, including new technologies such as single-cell multi-omics and artificial intelligence. Lastly, the discussion is summarized in Section 7, which provides conclusions and a prospective view of the process of integrating precision oncology in global health systems.

2. The Molecular Toolbox: Technologies Powering Profiling

The realization of precision oncology is fundamentally dependent on a sophisticated suite of molecular diagnostic technologies. Moving beyond single-gene assays, the modern molecular toolbox enables a comprehensive, multi-dimensional characterization of a tumor's genomic, transcriptomic, epigenomics and proteomic landscape. This section highlights technologies that are capable of generating primary data and, therefore, enabling personalized clinical decision making [8] (Figure 2).

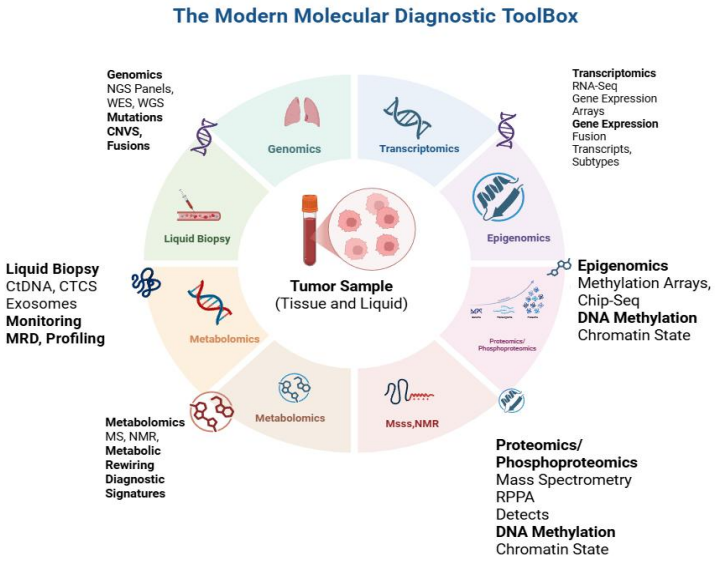

Figure 2. The molecular toolbox for precision oncology.

2.1 Genomic Interrogation

Genomic analysis is the main method for clinical tumor profiling. It identifies somatic changes in the DNA. NGS has made the analysis more comprehensive, affordable, and therefore, clinically feasible.

2.1.1 NGS: Panels, Whole-Exome (WES), and Whole-Genome Sequencing (WGS)

NGS can be used to parallel sequence millions of DNA fragments. There are three major methods used in clinical oncology, and each of them has its benefits. Targeted Gene Panels are the tool of routine care, which is aimed at a set of curated genes (50-500+) with clinical actionability. They provide high depth of coverage (>500x), which allows sensitive detection of low-frequency variants within small tissue samples, short turnaround times, and easy interpretation. Their weakness lies in the fact that they do not allow finding some new biomarkers beyond the scope of the panel [9]. WES sequences protein-coding regions of the genome (approximately 1-2 percent) as an equilibrium between discovery and cost. It can be useful in the detection of rare or unexpected actionable mutations, the definition of TMB, and research. Nevertheless, its reduced depth will fail to detect subclonal mutations, and it has a more complicated clinical interpretation. WGS is the most elaborate, which involves sequencing the whole genome. It is very good at identifying structural variations, non-coding variations, and intricate rearrangements with a lot of precision. Though previously prohibitive, the costs are falling, and WGS has become a potent instrument in the study of cancer etiology and the discovery of new drivers, though its more routine clinical application outside of panels and WES remains to be determined. Targeted NGS panels are the current standard of care for molecular profiling in a variety of tumor types, including non-small cell lung cancer (NSCLC), breast cancer, colorectal cancer, and ovarian cancer, with associated diagnostic approvals from regulatory authorities such as

the FDA. WES and WGS, while analytically powerful, are generally confined to research programs and select academic facilities because to the expense, turnaround time, and interpretive difficulty, and are not yet commonly indicated in standard clinical guidelines [10].

2.1.2 Key Actionable Genomic Alterations (e.g., EGFR, ALK, BRAF, BRCA, KRAS, PIK3CA)

Genomic profiling has clinical value based on the detection of alterations that have direct therapeutic relevance. These actionable changes can be divided into some categories: (1) Sensitizing mutations that indicate response to targeted inhibitors (e.g., *EGFR* exon 19 deletions in NSCLC, a response to osimertinib; *BRAF* V600E mutations in melanoma/CRC, response to dabrafenib/trametinib). (2) Loss-of-function mutations in tumor suppressor genes that make them susceptible to treatment, most notably BRCA1/2 mutations resulting in homologous recombination deficiency (HRD) and sensitivity to PARP inhibitors [11]. (3) Targeted oncogenic drivers could be both direct and indirect, such as targeting KRAS G12C mutations with the use of sotorasib and adagrasib. (4) Resistance mutations that can influence future treatment choice (e.g., EGFR T790M mutation). In addition, other important areas are being explored, for example, tumor-agnostic markers (high TMB, MSI-H) that predict response to immunotherapy regardless of cancer type [11] (Table 1).

Table 1. Examples of actionable genomic alterations and targeted therapies.

Gene / Alteration	Cancer Type (Example)	Therapeutic Class	Example Agent(s)	Key Trial (NCT no)	Objective Response Rates (ORR)	Level of Evidence	Refs.
EGFR sensitizing mutation (e.g., exon 19 del)	Non-Small Cell Lung Cancer (NSCLC)	Tyrosine Kinase Inhibitor (TKI)	Osimertinib, Erlotinib	FLAURA (NCT02296125)	~80% ORR (Osimertinib)	FDA-approved (1st/3rd line)	[12]
BRCA1/2 pathogenic mutation (somatic/germline)	Ovarian Cancer, Breast Cancer	PARP Inhibitor	Olaparib, Talazoparib	SOLO-1 (NCT0184498); OlympiAD (NCT0200062)	~60% ORR (Talazoparib/Olaparib in BRCA-mutant disease)	FDA-approved (maintenance / treatment)	[13]
NTRK1/2/3 gene fusion	Any Solid Tumor (Tumor-Agnostic)	TRK Inhibitor	Larotrectinib, Entrectinib	NAVIGATE (NCT0257643); STARTRK-2 (NCT0256826)	~75%-80% ORR	FDA-approved (tumor-agnostic)	[13]
BRAF V600E/K mutation	Melanoma, Colorectal Cancer	BRAF/MEK Inhibitor Combination	Dabrafenib + Trametinib	COMBI-d (NCT0158464); COMBI-v (NCT0159790)	~64%-69% ORR	FDA-approved	[14]
High TMB (≥10 mut/Mb)	Any Solid Tumor (Tumor-Agnostic)	Immune Checkpoint Inhibitor	Pembrolizumab	KEYNOTE-158 (NCT0262806)	~29% ORR	FDA-approved (tumor-agnostic)	[15]
KRAS G12C mutation	NSCLC, Colorectal Cancer	KRAS G12C Inhibitor	Sotorasib, Adagrasib	CodeBreaK100 (NCT0360088); KRYSTAL-1 (NCT0378524)	~37%-43% ORR in NSCLC	FDA-approved (NSCLC)	[15]
ALK rearrangement	NSCLC	ALK Inhibitor	Crizotinib, Alectinib	ALEX (NCT0207584)	~70%-82% ORR	FDA-approved	[16]
HER2 amplification	Breast Cancer, Gastric Cancer	Monoclonal Antibody / ADC	Trastuzumab, Trastuzumab Deruxtecan	DESTINY-Breast03 (NCT0352911); ToGA (NCT0104140)	~60%-80% ORR	FDA-approved	[16]

2.1.3 Copy Number Variations and Gene Fusions Detection and Significance

Alterations in genes are not confined only to single nucleotide alterations. Copy number alterations, both amplifications and homozygous deletions, have significant roles in carcinogenesis. HER2 amplification in breast and gastric cancers has been well-characterized for therapeutic targeting through Trastuzumab, while CDKN2A deletion occurs in multiple tumors. Copy number alterations can be detected using NGS or WGS techniques that analyze sequencing data based on coverage depths. Furthermore, gene fusions generated by chromosomal abnormalities create novel cancer fusion proteins and can act as potential targets for therapeutic intervention. Gene fusions such as the BCR-ABL1^{+} gene in CML, the EML4-ALK gene in NSCLC, and NTRK fusions in various cancers have been described. Gene fusions can be detected using DNA-based NGS that detects fusion genes through the detection of genomic breakpoints; however, intronic regions cannot be covered. RNA-based NGS and the use of platforms like NanoString detect fusion genes based on expressed RNA sequences [17].

2.2 Beyond the Genome: Multi-Omic Layers

Genomics serves as the main source of information. However, it is the functional state of a tumor that is determined by downstream molecular layers. Bringing together different types of omics data allows one to see tumor biology more thoroughly [18].

2.2.1 Transcriptomics: RNA-Seq and Gene Expression Signatures for Subtyping and Prognosis

Transcriptomics, through RNA sequencing (RNA-seq) quantifies the expression of all genes. Clinically, it is necessary in: (1) Validating gene fusions identified at the DNA level and new, expressed fusions. (2) Molecular subtyping: Dividing tumors into biologically distinct subsets with prognostic and predictive properties (e.g., the PAM50 intrinsic subtypes of breast cancer, consensus molecular subtypes of colorectal cancer). (3) The creation of predictive signatures: Expression-based commercial assays such as Oncotype D, X and MammaPrint are used to measure recurrence risk and inform the choice of chemotherapy in early breast cancer. New uses are the measurement of immune cell infiltration and anticipation of immunotherapy reaction [19].

2.2.2 Epigenomics DNA Methylation and Histone Modification Profiling

Epigenetic changes involve DNA methylation and histone modification without changing the DNA sequence to control the expression of genes. DNA methylation profiling, commonly with either array based or sequencing-based methods of bisulfate conversion, has large clinical utility. It is an effective diagnostic aid in the classification of central nervous system tumors (e.g., regarding the WHO classification) and cancers of uncertain primary origin and is more accurate than the traditional techniques. Loss of tumor suppressor genes can also be caused by aberrant promoter region methylation (e.g., MLH1 in Lynch syndrome). Besides, there is an emerging investigation of genome-wide epigenetic clocks and of methylation signatures as biomarkers to predict cancer risk, prognosis, and treatment responsiveness [20].

2.2.3 Proteomics & Phosphoproteomics MS-Based Analysis of Functional Signaling Hubs

Proteomics, which is normally performed by mass spectrometry (MS) or reverse-phase protein arrays, determines the abundance of proteins and post-translational changes (e.g., phosphorylation). This is essential since the levels of mRNA do not fidelity with its functional protein activity. Phosphoproteomics measures activated signaling pathways by measuring phosphorylated kinases and their targets, giving a direct measurement of oncogenic signaling flux (e.g., PI3K/AKT, MAPK pathways). This functional understanding can detect prevailing pathways of drivers, rationalize resistance to specific therapies and nominate combination approaches. Although it is now more common in studies, targeted proteomic panels are being transferred to the clinic to confirm the results of genomic studies and inform therapy [21].

2.2.4 Metabolomics A Tumor Metabolic Lens as a Diagnosis and Therapy

Metabolomics, which is based on MS or nuclear magnetic resonance (NMR) spectroscopy, measures small-molecule metabolites in a tumor. Metabolic environment is an active product of the genomic, transcriptomic, and proteomic activity and environmental limitations. Specific metabolic rewiring (i.e. Warburg effect, altered glutamine metabolism) generates specific metabolic signatures that can be used as diagnostic biomarkers (measured in blood or urine) and as therapeutic targets. In addition, metabolomics could also help unlock the process of drug resistance and sensitivity, which presents a new perspective on tumor biology and work to create therapeutic approaches that play on the metabolism of vulnerabilities [22].

2.3 The Minimally Invasive Revolution: Liquid Biopsy

Liquid biopsy, the analysis of tumor-derived material in bodily fluids, primarily blood represents a paradigm shift by enabling repeated, minimally invasive assessment of cancer.

2.3.1 Circulating Tumor DNA (ctDNA): Profiling, Monitoring and MRD Detection

ctDNA refers to the small and fragmented pieces of DNA that tumor cells release into the bloodstream. Ultra-sensitive NGS panels (less than 1% variant allele frequency) can be used to analyze ctDNA for three major clinical applications:

Comprehensive Profiling: Liquid biopsy of blood can detect the same actionable genomic alterations as those identified in a tissue biopsy. This approach is particularly valuable when tissue biopsy is limited or not feasible. However, its sensitivity may vary by tumor type and overall disease burden.

Longitudinal Monitoring: Clonal evolution and acquisition of new resistance mutations, such as EGFR *T790M* and *ESR1* mutations by serial ctDNA for tracking (weeks to months) before clinical or radiographic changes and early treatment intervention. MRD Detection: After surgery or chemoradiation, a positive test for ctDNA (MRD-positive) is a very specific sign of an impending relapse and might be used to make the decision about adjuvant therapy. In particular, tumor-informed (e.g., Signatera) patient-specific assays have a very high sensitivity for this use [23]. Despite the significant clinical applications of the technique, there exist certain biological and technical limitations of liquid biopsy. The sensitivity of the ctDNA detection process depends on the burden of the tumor, the degree of vascularity of the tumor, and its anatomic site, thus making it harder to detect early stage and low-burden tumors. Some types of cancer, for example glioma and mucinous neoplasms, demonstrate a limited capability for ctDNA release because of biological obstacles, such as the presence of the blood-brain barrier. Moreover, one

of the main challenges faced by scientists is the differentiation between tumor-specific and CHIP mutations, which occur as the consequence of mutations in the age-related *DNMT3A, TET2*, and *ASXL1* genes [24].

2.3.2 Circulating Tumor Cells (CTCs) and Exosomes

CTCs are intact cancer cells found in the bloodstream. Their counting is an officially accepted prognostic indication in metastatic breast, prostate, and colorectal cancers. Besides cell counting, nowadays platforms allow the molecular profiling of captured CTCs (genomic, transcriptomic) and even ex vivo culture for drug sensitivity testing, thus "functional biopsy." Extracellular vesicles (EVs), including exosomes, are also part of these and are lipid-bound nanoparticles that come from cells and carry proteins, nucleic acids (DNA, RNA), and lipids. They are very common and resistant in biofluids. EVs study is a rapidly progressing area that holds the promise of giving a comprehensive multi-omic tumor snapshot, including phosphoprotein signaling states and miRNA profiles that could be used as diagnostic and predictive biomarkers [25].

2.3.3 Comparative Utility: Complementary Roles of Tissue and Liquid Biopsy

Tissue and liquid biopsies can be seen as complementary modalities. Now tissue biopsy is considered the gold standard for primary diagnosis because it gives histopathological context, tumor purity, and spatial (e.g. PD-L1 immunohistochemistry (IHC), tumor microenvironment architecture) information. However, the drawbacks of tissue biopsy are that it is invasive, there is a risk of sampling bias due to intra-tumor heterogeneity, and it cannot be done repeatedly. Liquid biopsy can capture a systemic, real-time, total disease-burden snapshot, including the heterogeneity of multiple sites of metastasis. Besides its superiority in serial monitoring and MRD detection, it is also useful in profiling when the tissue is not available. A combined approach (tissue for the initial comprehensive profiling and liquid biopsy for longitudinal surveillance and monitoring the development of resistance) is a wise solution to the oncology strategy in many cases [26].

3. From Biomarker to Therapy: Clinical Translation of Molecular Data

For molecular profiling, its greatest worth is realized when it guides and changes clinical management directly. Here, we describe the use of biomarker data at various stages of cancer care, from improving initial diagnosis, to choosing targeted therapies, immunotherapy optimization, and dealing with treatment resistance, which is bound to happen [27].

3.1 Refining Diagnosis and Stratification

Molecular data has essentially revolutionized traditional methods of diagnosis classification through the addition of an objective biological component that complements risk assessment and therapy prediction.

3.1.1 Molecular Reclassification of Histological Entities

One of the most prominent genomic studies, The Cancer Genome Atlas (TCGA), has demonstrated that cancers having a similar appearance can be further categorized into molecular subtypes that differ significantly in clinical phenotypes and therapeutic susceptibility. One of the main causes that has facilitated the integration of molecular features into the diagnostic standards is this very issue. For example, IDH mutation status and 1p/19q co-deletion are now more prognostically relevant in the classification of diffuse gliomas than their histological features. In addition, the intrinsic subtypes in breast cancer (Luminal A/B, HER2 enriched, Basal-like) are more predictive regarding the selection of systemic therapy than grading or staging. Furthermore, the entire spectrum of molecular characterization up to DNA methylation is considered the standard in diagnosis for tumors and cancers of unknown primary origin (CUP), which helps to determine the tissue of origin and plan site-specific treatment [28].

3.1.2 Differences Between Prognostic and Predictive Biomarkers in Relation to Clinical Utility

The major distinction for clinical translation lies in prognostic and predictive biomarkers. While the prognostic biomarker can offer information about the course of disease, which includes how aggressive the disease would be in terms of its chances of recurrence, both with and without therapy, the example is the 70 gene signature (MammaPrint) for breast cancer and TP53 mutations for the majority of cancers. However, a predictive biomarker determines the chances of individual patients responding to therapy in a certain way. Examples include EGFR mutations for determining sensitivity to tyrosine kinase inhibitors in NSCLC and BRCA1/2 mutations for identifying those patients sensitive to PARP inhibition. Both of the prognostic and predictive biomarkers are equally powerful from a clinical perspective. The importance of this differentiation cannot be understated as it determines how one utilizes tests as well as how information is provided to the patient [29].

3.1.3 Integrative Diagnostics: Correlation between Molecular, Pathological, and Imaging Characteristics

The next phase in diagnostic involves using integration methods that include multi-scale data analysis. Radiomics refers to the technology used in analyzing different quantitative features on a large scale using imaging tools such as CT, MRI and PET scans, which are associated with molecular phenotypes (radiomics can be used to detect EGFR mutations in patients with lung cancer). Pathomics involves the use of computerized analysis to digitize slides of pathological histopathology. It enables AI to accurately predict genomic changes using tissues stained by H&E alone. Combination of all these techniques including "omics" technologies is known as pan-omics and is meant to create a fully mapped picture of the tumor [30].

3.2 Targeted Therapeutic Strategies

One of the most distinctive aspects of precision oncology is that there has been a close linkage of the marker at the molecular level with a certain drug, and it has entirely transformed the treatment approach for certain patients.

3.2.1 Therapies Directed by Genotype: Kinase Inhibitors, Monoclonal Antibodies, and ADCs

Targeted therapies are designed to disrupt certain molecular components that play a crucial role in tumor survival and growth. Small molecule inhibitors (such as osimertinib for EGFR mutant NSCLC, vemurafenib for BRAF V600E mutant melanoma) act by penetrating the cell and disrupting oncogenic signaling pathways. Monoclonal antibodies (such as trastuzumab against HER2 and cetuximab against EGFR) bind to cell surface receptors, thereby inhibiting signaling while simultaneously stimulating immune response. Antibody-drug conjugates (ADCs) are a powerful advancement that combine the targeting precision of a mAb with the cytotoxic release of chemotherapy (e.g., trastuzumab deruxtecan for HER2-low breast cancer, sacituzumab govitecan for TROP-2-expressing cancers). The clinical progression of these drugs is fundamentally linked to the simultaneous progression of a companion diagnostic (CDx) to identify the eligible biomarker-positive population [31].

3.2.2 Tumor-Agnostic (Histology-Independent) Treatments: A New Paradigm

It is the basic paradigm shift of moving away from tissue-based drug approvals towards genomics-based ones. In fact, such treatments are allowed solely on the basis that a particular molecular biomarker is present even if the tumor is of a different tissue of origin. Some of the most prominent cases are:

TRK inhibitors (larotrectinib, entrectinib) are used for any solid tumor harboring an *NTRK* gene fusion. Immune checkpoint inhibitors (pembrolizumab, dostarlimab) can be given to any tumor that is high in MSI (MSI-H) or has a mismatch repair deficiency (dMMR). The drug-device combination of pembrolizumab is used for tumors with high TMB (TMB-H, ≥10 mutations/megabase).

This is a radical departure from the old model in which the drug must be proven to work in each tissue type. It is a way of proving that one should target the most fundamental oncogenic driver mechanism and will have significant consequences for those with rare cancers or with cancers that were unconventional in the way they looked. However, the problem of biomarker testing being less accessible is still a challenge [32].

3.2.3 Companion and Complementary Diagnostics: Regulatory and Clinical Pathways

CDx refers to a medical device, frequently an in vitro diagnostic test, which is necessary to ensure the safe and effective usage of a corresponding drug. It can be used to determine the patients who are more likely to be responsive (e.g., VENTANA PD-L1 assay for pembrolizumab in NSCLC). Usually, regulatory agencies (FDA, EMA) approve the drug and CDx at the same time. A Complementary Diagnostic gives additional information that can be used to decide on a treatment, but it is not a drug administration requirement. Since biomarker-drug relationships are becoming increasingly complex, with multiple biomarkers being used to guide a single therapy (e.g., ALK testing by IHC, FISH, or NGS), the clinical pathway relies on validated standard-of-care testing algorithms established by professional guidelines to ensure that patients receive appropriate, biomarker-directed therapy [33].

3.3 Informing Immuno-Oncology

Immunotherapy has revolutionized cancer treatment and molecular profiling is essential for detecting responders, non-responders, and new immunotherapeutic targets.

3.3.1 Predictive Biomarkers for Immune Checkpoint Inhibition (PD-L1, TMB, MSI, dMMR)

Despite remarkable successes, only a subset of patients responds to immune checkpoint inhibitors (ICIs), necessitating predictive biomarkers. PD-L1 expression by IHC has been the initial widely used biomarker, although it is not perfect, and its predictive value varies across different cancer types and assay platforms. TMB-H leads to the increased production of neoantigens, thereby rendering tumors more immunogenic and susceptible to ICIs. MSI-H/Mismatch Repair Deficiency (dMMR) causes a hypermutated phenotype and is a very strong pan-cancer predictor of ICI response. These biomarkers are increasingly used in combination, as they provide non-redundant information about the tumor-immune interface [34]. Other than the standard biomarkers such as PD-L1 and TMB, the efficacy of the treatment regimen using immune checkpoint inhibitors is highly influenced by the tumor microenvironment (TME). The TME refers to interactions between tumor cells, stromal fibroblasts, endothelial cells, suppressive myeloid cells, and tumor-infiltrating lymphocytes to govern immune responses and treatment responses. Immunosuppressive factors like TGF-β, IL-10, and VEGF contribute to immune dysfunction and inhibition, resulting in resistance of the tumor towards an immune response. The constant presentation of antigens in the TME triggers T-cell exhaustion, which is marked by the expression of various negative regulatory receptors including PD-1, CTLA-4, LAG-3, TIM-3, and TIGIT, alongside epigenetic and metabolic changes. The process is mainly associated with "cold" tumors with minimal T-cell infiltration and interferon signaling. Thus, new approaches for treatment entail converting the cold tumors to hot tumors by means of combinations of treatments including radiotherapy, anti-angiogenic drugs, oncolytic viruses, STING agonists, epigenetic therapies, and personalized neoantigen vaccination (Table 2).

Table 2. Key predictive biomarkers for immune checkpoint inhibitors.

Biomarker	Primary Measurement Method	Predictive Value & Rationale	Clinical validation level	Predictive value and rationale	Key Limitations	Refs.
PD-L1 Expression	Immunohistochemistry (IHC)	Higher expression correlates with increased likelihood of response in many cancers (e.g., NSCLC); reflects immune checkpoint presence.	Clinically validated	Higher PD-L1 expression corresponds with better response to ICIs (e.g., NSCLC, melanoma), indicating immune checkpoint activation.	Cut-off variability between assays and cancer types; imperfect sensitivity/specificity; intratumoral heterogeneity.	[35]
Tumor Mutational Burden (TMB)	Next-Generation Sequencing (NGS)	High TMB (≥10 mutations/megabase) correlates with increased neo-antigen load and improved response to ICIs across multiple tumors.	Clinically validated	High mutational load boosts neoantigen production and immunogenicity.	Lack of standardized calculation, influenced by cancer type and panel size; high cost.	[35]
Microsatellite Instability (MSI) / Mismatch Repair Deficiency (dMMR)	PCR (MSI) or Immunohistochemistry (dMMR)	Defective DNA repair leads to hypermutation and high neoantigen burden; robust tissue-agnostic predictors of ICI response.	Clinically validated	Defective mismatch repair causes hypermutation and high immunogenicity.	Low prevalence in most common cancers (except Lynch syndrome–associated tumors).	[36]
Gene Expression Profiling (e.g., T-cell inflamed signature)	RNA Sequencing (RNA-Seq) or NanoString	Quantifies immune cell infiltration and interferon-γ signaling within the tumor microenvironment (TME).	Emerging clinical biomarker	reflects the immune-inflamed TME and T-cell activation.	Not yet standardized for routine clinical use; requires high-quality RNA.	[36]
Tumor-Infiltrating Lymphocytes (TILs)	Histopathology (H&E) or IHC	Dense lymphocytic infiltration is associated with improved response to immune checkpoint inhibitors.	Emerging/ supportive biomarker	Pre-existing antitumor immune response	Qualitative or semi-quantitative; observer-dependent assessment.	[37]

3.3.2 Neoantigen Prediction and Personalized Cancer Vaccines

Neoantigens are T cell-recognizable tumor-specific peptides that occur because of somatic mutations. Predicting neoantigens with the help of computational pipelines that combine genomic and transcriptomic data can be based on such factors as the expression of mutant alleles, the affinity of peptide-MHC interactions and the probability of TCR recognition. This forecast is motivating personalized cancer vaccines, either mRNA-based or peptide-based. They are tailor-made de novo vaccines to induce a T-cell response in a patient to their own tumor neoantigen repertoire. Innate immunogenicity and clinical activity in preclinical phase clinical trials for melanoma and other cancers have demonstrated promise, approaching the ultimate goal of personalized immunotherapy [38].

3.3.3 Immune Microenvironment Profiling: Transcriptomic and Spatial Signatures

Relative abundance of populations of immune cells (e.g., by using tools like Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT)) can be deconvoluted by bulk gene expression profiling of tumor RNA, which can produce an immunophenotype. ICI response is associated with signatures of T- cell inflammation or IFN-g signaling. Nonetheless, it is the spatial transcriptomics and multiplex immunohistochemistry/immunofluorescence (mIHC/IF) that are transforming this domain by maintaining the architecture. Such technologies enable researchers to map the exact geographic location and functional condition of immune cells compared to tumor cells, stromal elements, and vasculature. Important spatial measures, including the distance of CD8+ T cells to tumor cells or tertiary lymphoid structure, are becoming potent outcome and resistance to immunotherapy predictors [39]..

3.4 The Challenge of Resistance: Guiding Sequential Therapy

The development of therapeutic resistance is nearly universal, and molecular profiling is essential for understanding its mechanisms and guiding subsequent lines of therapy.

3.4.1 Mechanisms of Acquired Resistance to Targeted and Immunotherapies

Resistance can be categorized as on-target (modifications of the drug target itself, e.g., *EGFR T790M* or *C797S* mutations after osimertinib) or off-target (activation of bypass signaling pathways, e.g., *MET* amplification or phenotypic transformation to small cell lung cancer after EGFR inhibitor therapy). In immunotherapy, resistance mechanisms are more diverse and include: loss of neoantigens, defects in antigen presentation (e.g., *B2M*

mutations), upregulation of alternative immune checkpoints (e.g., TIM-3, LAG-3), and recruitment of immunosuppressive cell populations (Tregs, M2 macrophages). Profiling at progression is critical to identify the dominant resistance mechanism [40].

3.4.2 The Role of Serial Liquid Biopsy in Tracking Clonal Evolution

Progression Tissue re-biopsy can be problematic. Serial liquid biopsy using ctDNA analysis is a dynamic and non-invasive technique to track clonal evolution on a real-time basis. It is able to identify the development of resistance mutations several weeks or months prior to radiographic progression and proactively switch the therapy. In addition, it demonstrates the polyclonal character of resistance and the development of many competing resistant subclones at the same time. This insight underlies non-directional sequential monotherapy and justifies the reason as to why combination approaches or newer bispecifics, are engineered to conquer various resistance mechanisms in the first instance. Recent developments in targeted therapies have been focused on overcoming acquired resistance via generation four inhibitors as well as protein degradation approaches. The fourth generation of TKI therapy for C797S-mediated drug resistance, SHP2 inhibitors, bispecific antibodies, and Proteolysis-Targeting Chimera(s) (PROTACs) represent novel tools for combating resistant clones of tumor cells. Nevertheless, tumors may counteract treatment via molecular rewiring and adaptation by activating parallel pathways such as PI3K/AKT/Mechanistic Target of Rapamycin (mTOR), MAPK, MET, and YAP/TAZ signaling. Other adaptations include Epithelial–Mesenchymal Transition (EMT), cell lineage plasticity, epigenetic rewiring, and reprogramming of the TME. In this regard, it becomes evident that the development of resistance is more complex than the presence of secondary mutations [41].

3.4.3 Strategies to Overcome or Prevent Resistance

Based on molecular profiling, several other approaches are currently undergoing clinical development: (1) Rational Combination Therapies: Simultaneously combining an initial driver with a well-understood bypass pathway (e.g., EGFR + MET inhibition). (2) Intermittent Therapy / Therapy Holidays To reduce selection pressure and enable the sensitive clones to come back, perhaps re-sensitize the tumor to the initial medication. (3) Development of Next Generation Inhibitors: This refers to the development of resistance mechanisms discovered in various tumor cell lines (for example, the 4th generation EGFR inhibitors C797S). (4) Adaptive Therapy Models: These require a regular analysis of tumor cells based on their molecular characteristics (using liquid biopsies) to determine the times when therapy should be administered or not, thus ensuring that the dominant clones are controlled over the long term and not aggressive resistance achieved through maximum cell kill [42].

4. Navigating Complexity: Implementing Profiling in Clinical Decision-Making

Generation of molecular data marks only the starting point in the complex process of making precise use of the information to devise appropriate treatment strategies for the patient; interpretation of the data and application thereof represent the main hurdles in the field. The following paragraphs will elaborate on important pathways involved in achieving this goal.

4.1 The Interpretation Imperative

A genomic mutation does not necessarily translate into a treatment recommendation. The translation is accomplished through the complex process of clinical interpretation.

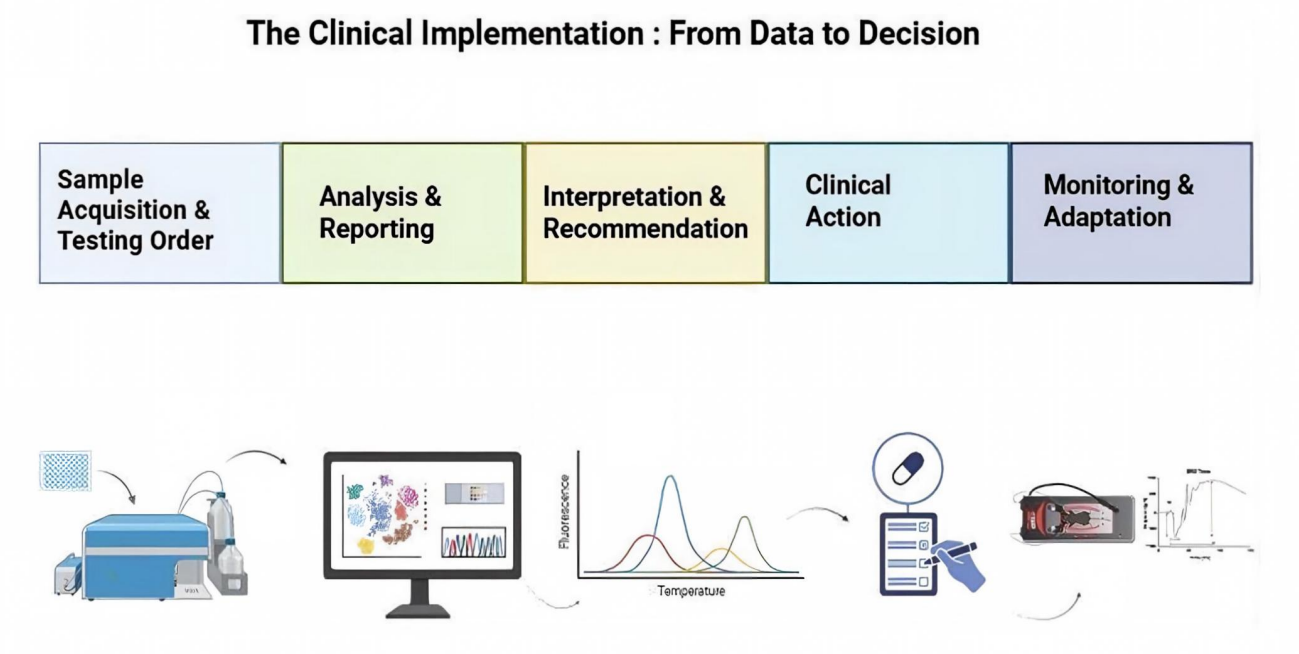

Figure 3. Clinical workflow in precision oncology from data to decision.

Figure 3 shows a step-by-step process of five stages involved in converting biological specimens into a practical clinical decision-making framework. First is specimen acquisition and diagnosis, followed by specimen processing and data interpretation, and later, clinical decision-making and treatment. In other words, the patient is being monitored throughout the entire process. This technology uses laboratory equipment, computer analysis, and clinical knowledge to foster evidence-based care.

4.1.1 Variant Annotation

The initial one is to categorize all variations. The changes that have a clear and evidence-based relationship with a particular form of therapy or clinical outcome are actionable changes. They are frequently divided into layers (e.g., Level 1: FDA-recognized biomarker of an approved drug in that specific cancer type; Level 2: standard-of-care biomarker of an approved drug in another cancer type). Most broad panel testing results are represented by VUS. Such are changes whose biological or clinical effects are not known and should not be applied to inform therapy outside clinical trials. Another important element that should be critically considered and is mostly ignored is the recognition of potentially germline findings. Identification of pathogenic variants in such genes as BRCA1/2, TP53, and mismatch repair genes in tumor tissue may represent a hereditary cancer syndrome and lead to a genetic counselling referral and confirmatory germline testing, with implications to both the patient and family [43].

4.1.2 Leveraging Evidence-Based Knowledgebases (OncoKB, CIViC) and AI-Powered CDS Tools

Manual interpretation cannot be supported by the size and speed of incoming data. A curated knowledge base is used by clinicians and molecular pathologists. OncoKB is an open-access, high-volume, precision oncology database that annotates therapeutic, prognostic, and diagnostic evidence of each genomic alteration and provides a clinical actionability level. CIViC (Clinical Interpretation of Variants in Cancer) is an open-source project available to the community and crowdsources evidence and interpretations.These are increasingly incorporated into bioinformatics workflows and Clinical Decision Support (CDS) systems within clinical practice.Next-generation CDS systems are artificial intelligence (AI) based systems that do not just summarize findings of the knowledge base but also query the entire molecular profile of the patient in the original literature, clinical trial databases, and real-world evidence to provide ranked therapeutic hypotheses, thus enhancing the decision-making of clinicians [44].

4.1.3 Standardized Reporting Frameworks for Clinicians

Molecular pathology reports are the interface used to convey the complex information to the treating oncologist. Such reports have to be succinct, concise, and concise. Guidelines for reporting include: a clear executive summary that highlights the actionable findings; tiering or grading actionability levels of clinical findings; parsimonious description of actionability of somatic and potentially germline findings in clinical settings; and implications for therapeutic decisions (such as "This PIK3CA H1047R mutation is a Tier 1 biomarker that will help in using alpelisib in combination with fulvestrant for HR+/HER2- metastatic breast cancer, according to the SOLAR-1 trial). Standards for reporting are exemplified by the guidelines issued by the Association for Molecular Pathology (AMP)/ American Society of Clinical Oncology (ASCO)/ College of American Pathologists (CAP) [45].

4.2 The Multidisciplinary Molecular Tumor Board (MTB)

The Molecular Tumor Board is an interdisciplinary discussion group that forms the bedrock of complex molecular information interpretation and personalization of treatment, especially for cancer patients with no other options remaining in standard treatments.

4.2.1 Essential Structure

The Medical Tumor Board (MTB) deliberations consist of a multi-disciplinary and unified process in which medical oncologists, pathologists, geneticists, and bioinformaticians collaborate within one decision-making model. A successful MTB reflects a highly coordinated clinical microenvironment. Some of the individuals include, for instance: medical oncologists who are responsible for considering past treatment history, performance status, and therapeutic aims of the patients; molecular pathologists who will comment on the test’s analytical validity and biological variants; clinical geneticists or genetic counselors who provide information about the possibility of inherited diseases that may have arisen from genome analysis; and bioinformaticians or data scientists who provide information about data-processing methods and their limitations. The expertise of pharmacologists, immunologists, radiation oncologists, and clinical trial coordinators is also brought in to contribute to the case-specific discussions [46].

4.2.2 Function and Impact on Treatment Planning and Clinical Trial Matching

The MTB has a list of major functions: (1) Reconciling Discrepant Findings: Reconciling conflicting assays or molecular and clinical evidence. (2) Case Interpretation: Talking about VUS, new mutations or complicated mutational signatures to evaluate their actionability. (3) Creating Therapeutic Recommendations: Proposing evidence-based standard therapies, off-label use of targeted agents (based on molecular rationale), or specific clinical trials. Research has demonstrated that the review of the MTB directly results in the change of the management in 10-50 per cent cases, and a substantial part of the recommendations included matching the patients to the genotype-selected clinical trials, which could frequently be assisted by the real-time integration of trial databases (e.g., ClinicalTrials.gov) during the discussion of the MTB [47].

4.2.3 Case Studies Illustrating MTB-Driven Patient Management

Case A (Tumor-Agnostic Action): A patient with a rare carcinoma of unknown primary, refractory to chemotherapy, undergoes comprehensive genomic profiling (CGP). MTB detects high tumor mutation burden (TMB-H). Pembrolizumab has been FDA-approved based on tissue-agnostic treatment of TMB-H tumors; thus, immunotherapy is suggested by the MTB, resulting in a durable partial response [48].

Case B (Resistance Overcome): An individual with EGFR mutation-positive NSCLC develops resistance to osimertinib treatment. ctDNA from the plasma shows that an acquisition of MET amplification has occurred. After analyzing the literature on EGFR/MET co-inhibition treatment, the MTB suggests participation in a clinical trial evaluating the combination [49].

Case C (VUS to Action): An example would be an MSI-H colon cancer metastasis, which carries a VUS in the POLE gene coding for DNA polymerase. After the MTB discussion and expert opinion, there was consensus that the ultra-hypermutated profile is due to some mutations in POLE; hence, acting like MSI-H. Therefore, it can be rationalized that immunotherapy would work on this patient. This was successful, and it was later discovered that the variant is indeed pathogenic [50].

4.3 Operationalizing Precision Oncology

Precision medicine has to be thoroughly incorporated into sustainable, equitable clinical workflows if it is to leave the confines of the academic MTB and become standard care.

4.3.1 Integration into Clinical Workflow: From Test Ordering to Treatment

The smooth process flow becomes very important. Testing Indications and Order: Reflex testing algorithms (such as auto-testing for EGFR in any lung adenocarcinoma) and clinical decision support integrated into the EHR system will help in choosing the right test. Pre-analytic Stage: Control quality of the standardized process of tissue collection, fixation, and isolation of nucleic acids. Tracking & turnaround time (TAT) management: Use tracking systems to monitor test processing, since in the clinic decisions need to be made on time. Reporting and Results Integration: Standardized reports are automatically generated in the Electronic Health Record (HER) and default notifications are sent to the physician making the order. Decision Points: Providing access to reviewing MTB and clinical decision support transparency [51].

4.3.2 Logistics Barriers: Time to Receive Results, Expense, Payment, and Accessibility

Logistical issues will constantly occur and may potentially lead to a fair process being hindered. The TAT time for large panels may range from 2 to 4 weeks which might be quite long in case the patient suffers from a quickly progressing condition. Cost and Reimbursement will present considerable problems as reimbursement organizations often have limited reimbursement for large panels especially in cases where the use of large panels is off-label, either in early-stage or based on molecular data. Inaccessibility would become a huge problem since patients from a community setting, rural areas, and other resource-poor regions would lack access to the procedure. The solutions to these include advocacy on wider insurance coverage, formulating cost-effective testing strategies and the need to initiate public health programs to curb disparities [52].

4.3.3 Educating Clinicians and Empowering Patients

Shared Decision-Making and Consent. Both sides of the clinic will require education to be successfully implemented. Clinician Education should not be limited to oncologists only but should also cover the surgeons, pathologists, and primary care physicians. They should be literate in molecular terms, interpretation of tests, and the constraints of genomic data. In patients, it needs to be performed with the focus on pre-test counseling to discuss the possible advantages (discovery of a treatment target), risks (incidental germline findings, psychological effects of VUS), and limitations of testing. Data sharing in research should be included in the informed consent. The first recommendation is shared decision-making in situations where a molecular discovery indicates an off-label treatment or a clinical trial with questionable utility; the physician needs to make sense of the data, and the patient considers his or her own principles and risk-taking. Communication tools should be used effectively to close the gap between the complicated genomic data and patient comprehension [53].

5. Current Frontiers and Persistent Challenges

The promise of precision oncology was to some degree shadowed by multiple biological, technical, economic and ethical barriers. To overcome all these barriers is vital if we want to implement molecular technologies in practice.

5.1 Biological and Technical Hurdles

The biological complexity and technological requirements to measure this complexity become barriers to achieving perfect precision.

5.1.1 Tumor Heterogeneity, Evolution, and the Sampling Problem

Heterogeneity in tumors represents one of the biological challenges to precision oncology, arising because of the heterogeneity that may exist genetically, epigenetically, and phenotypically in and among tumors. Intertumoral heterogeneity describes how various subclonal populations may exist within a tumor and create regional differences in targetable mutations and vulnerabilities. Additionally, there is heterogeneity between primary and metastatic tumors, contributing to different molecular characteristics as well as differences in response to treatment. In a process called clonal evolution, cancer cells evolve under treatment pressures through selective

expansion of resistant clones, ultimately contributing to resistance and failure of the treatment method. Another challenge is represented by the sampling issue, whereby biopsies performed at one site will not provide a full representation of tumor heterogeneity [54]. However, these constraints can be overcome through the use of longitudinal sampling and multi-region sampling approaches, which include ctDNA testing. Nevertheless, tumor plasticity remains a major problem that poses as an obstacle in obtaining reliable and valid results. Another aspect to consider is tumor heterogeneity, where different regions within one tumor may have different genotypes. Thus, there may be cases where one tissue sample cannot provide enough information concerning the genotype of the cancer cell, which could lead to sampling error. This may cause a situation in which the detection of subclonal or spatially isolated variants may be underestimated due to the presence of such limitations, despite their clinical relevance in terms of drug resistance or disease progression. Moreover, tumor mutations change over time due to tumor progression and selection pressure from treatments, leading to inaccurate genotyping when using archived or static samples. Therefore, inadequate sampling can compromise the integrity of obtained data and negatively affect the accuracy of variant interpretation [55].

5.1.2 Analytical Validity

Standards, Bioinformatics Pipes, and Quality Control. It is a risky journey to go from the analysis of the tissue samples to a clinical report. Pre-analytical variables might play a crucial role in the result, including fixation time, ischemic time, and nucleic acid condition. In addition, wet lab methods and sequencing platforms differ between laboratories. Above all, bioinformatic analysis uses complicated pipelines in order to conduct alignments, variant calling, and annotation processes; even small differences in the pipeline used might generate different variant calls in various laboratories when analyzing the same sample. Quality control, proficiency testing (such as that conducted by CAP), and adherence to new standards (for example, SEQC2 project conducted by the FDA) should be carried out strictly; however, this is currently not the case [56].

5.2 Socioeconomic and Systemic Barriers

Constraints related to precision oncology are not only scientific but also economic and logistical, leading to substantial inequalities.

5.2.1 The Exorbitant Cost of Precision Oncology: Economics and Health System Challenges

Precision oncology delivers a high therapeutic impact while placing tremendous financial strain on health care systems owing to the high cost of molecular diagnostic tests, genomic analysis equipment, and targeted drugs. NGS technologies, especially those involving comprehensive multi-gene panels and whole-genome analysis, remain costly at scale when combined with repetitive analysis tools, such as ctDNA. Financial stress is further compounded by therapeutic costs in light of the fact that targeted drugs and immunotherapy are often administered on a regular basis and are priced highly per each dose. Payment strategies have not yet been optimized to incorporate biomarker and tumor-agnostic approaches into their logic, making it difficult to cover precision oncology treatments and ensure patient access. In other words, there is an increasing necessity for including cost-effectiveness criteria in precision oncology. Health Technology Assessment (HTA) approaches that incorporate genotypic information and real-world evidence are being developed; however, in absence of adequate cost management and reimbursement schemes, precision oncology will likely remain confined to well-endowed settings [57] (Table 3).

Table 3. Major challenges in the implementation of precision oncology.

Domain	Specific Challenge	Potential Consequences	Refs.
Biological & Technical	Tumor Heterogeneity & Evolution	Single biopsy may miss key molecular drivers; therapy resistance may develop, necessitating repeated sampling.	[58]
Biological & Technical	Analytical Standardization	Lack of uniform bioinformatics pipelines, variant calling, and reporting confounds data comparison and clinical interpretation.	[58]
Socioeconomic & Systemic	High Cost of Testing & Therapies	Creates financial toxicity for patients, strains healthcare systems, and limits access to precision oncology.	[59]
Socioeconomic & Systemic	Inequitable Access & Disparities	Widened gap in cancer outcomes based on geography, race, ethnicity, and socioeconomic status.	[59]
Socioeconomic & Systemic	Reimbursement & Policy Lag	Payers may not cover broad NGS panels or off-label use guided by molecular evidence, limiting patient access.	[60]
Socioeconomic & Systemic	Infrastructure & Capacity Gaps (especially in LMICs)	Lack of laboratories, bioinformaticians, and clinical genetics expertise prevents implementation in low-resource settings.	[60]
Ethical & Logistical	Interpretation of VUS & Incidental Findings	May lead to patient anxiety, inappropriate clinical action, or increased need for complex genetic counseling.	[61]
Ethical & Logistical	Data Privacy & Security	Genomic data is uniquely identifiable and immutable, raising substantial risks if breached.	[61]
Ethical & Logistical	Turnaround Time for Results	Delays of 2-4 weeks for NGS can impact treatment decisions for rapidly progressing patients.	[62]
Ethical & Logistical	Hope vs. Hype / Therapeutic Misconception	Overestimation of actionability can result in the use of ineffective therapies and patient disappointment.	[62]

5.2.2 Equity and Access: Combating Inequities Among Populations and Regions

Although technology is developing rapidly, availability of precision oncology depends on geographic location and social status. Advanced genetic infrastructure, bioinformatics, and clinical support decision-making technologies are available in rich countries, while poor and middle-income countries lack the necessary diagnostics and analysis. Among these deficiencies are insufficient numbers of NGS instruments, unskilled personnel, non-standardized genomic labs, and ineffective reimbursement policies. Even in adequately funded health care, there are still disparities between rural communities and people living in less affluent areas, who cannot undergo molecular analysis and obtain personalized treatment.Non-uniformity in global genomics, such as sequencing panels, analysis procedures, and reporting standards, is another issue that needs to be addressed to standardize clinical decision making. Non-uniformity in global genomics, such as sequencing panels, analysis procedures, and reporting standards, is another issue that needs to be addressed to standardize clinical decision making.To solve the above issues, a multi-level strategy should be used. Regionally specific and affordable gene panel testing will make it possible for genomic analysis to become more accessible, while open-source bioinformatics software and international data exchange will help avoid interpretation differences. In sum, implementation of precision oncology is possible only when technology, system improvement, and cooperation occur globally [63].

5.2.3 Infrastructure and Capacity Gaps, Especially in LMICs

Low- and middle-income countries (LMICs) are where the gap of implementation is the most severe. Obstacles are the prohibitive prices of treatments, far-near deficiency of reimbursement systems, and so on, and the inadequacies of infrastructure: unreliable electricity, insufficient sample transport systems, and a shortage of molecular pathology laboratories, bioinformatics and clinical genetics. On the one hand, it is recommended to use scaled-down, economical, and lean-based gene panels that target the most locally pertinent changes, which places the risk of building a two-level global structure of precision medicine. International cooperation, technology transfer, open-source bioinformatics tools, and localized training programs to meet the needs and cancer epidemiology are necessary to develop sustainable capacity [64].

5.3 Ethical and Data Governance Considerations

The collection and use of genomic data raise profound ethical questions that extend beyond the individual patient to society at large.

5.3.1 Data Privacy, Security, and Ownership in a Genomic Era

The genomic data is identifiable, unalterable, and predictive, which is the source of exceptional privacy concerns. Genomic and clinical information centers are valuable targets of hacking. The questions about the data ownership and stewardship remain unsolved: who owns this data: patients, hospitals, testing companies, or research consortia? Data sharing policies to be used in research need to strike a balance between the overall benefit of scientific advancement and individual liberty and privacy, usually with regard to broad consent procedures. Of potential concern to the patient is the risk of genetic discrimination, which, despite being prohibited in theory by laws such as the Genetic Information Nondiscrimination Act (GINA) in the U.S., is still a fear of the patient, especially when it comes to life insurance or long-term care insurance in most jurisdictions [61].

5.3.2 Ethical Dilemmas: Incidental Findings, VUS, and Hope vs. Hype

The clinical experience is ethically challenging. Incidental or secondary findings Incidental or secondary findings involve the discovery of a pathogenic germline variant in a cancer predisposition gene (such as TP53, PTEN) that is not relevant to the reason behind the primary testing (a duty to act) with complex genetic counseling and possible psychosocial harm. VUS present an ethical dilemma to the management of next day, reporting them can provoke anxiety and encourage inappropriate clinical response, and withholding them can go against transparency reporting. Lastly, is the so-called therapeutic misconception or the misconception of hope vs. hype. Patients and clinicians can be over-optimistic about the actionability of the molecular discovery, so they administer costly, toxic treatments that have limited evidence supporting their effectiveness. This puts a moral strain on clinicians to be honest on presentation of probabilistic findings, to manage expectations and to negotiate shared decisions in a profoundly uncertain environment, so that beneficence and non-maleficence are not adversely affected by the pursuit of precision [65].

6. The Future Landscape: Emerging Technologies and Directions

As precision oncology evolves, it is poised for its next phase through technologies that provide a level of detail and analytical capability never seen before, together with new means of gathering evidence and delivering care. In this part, we will explore what the future holds: prediction-based diagnoses, adaptive treatments, and proactive control over cancer [66] (Figure 4).

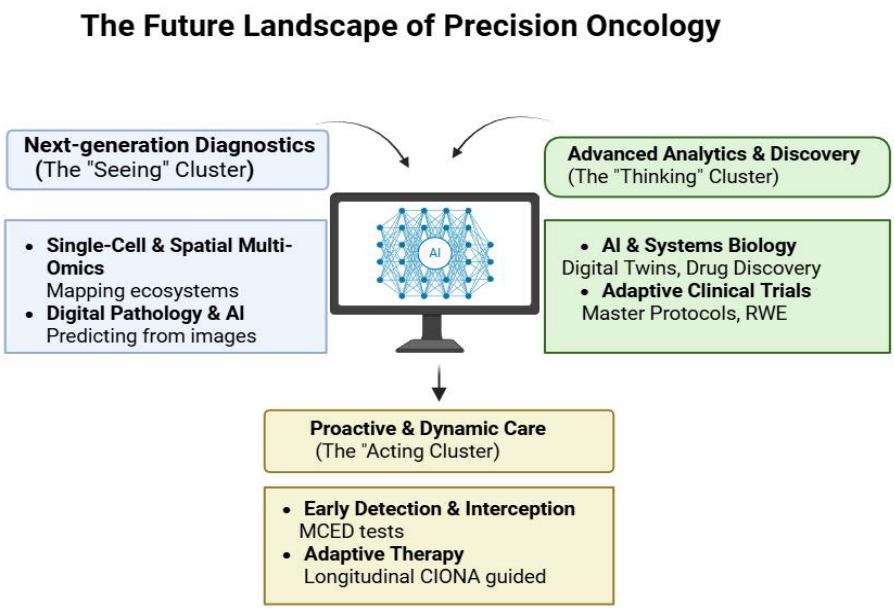

Figure 4. Challenges and future directions in precision oncology.

6.1 Next-Generation Technologies

New technologies are evolving from whole tissue analyses to unraveling the spatial organization and cellular heterogeneity of cancer tumors, unveiling biology that was concealed by averaging.

6.1.1 Single-Cell and Spatial Multi-Omics

Mapping Tumor Ecosystems Bulk DNA sequencing dilutes the signals obtained across millions of cells, thus failing to capture the great heterogeneity within a tumor. This problem can be solved using single-cell multi-omics such as scRNA-seq or scATAC-seq, where the genomics, transcriptomics, and epigenomics of individual cells are analyzed. Such approaches provide information regarding peculiar cell phenotypes, such as drug-resistant persistent cells or metastatic clones, as well as the accurate characterization of immune and stromal cell types within the TME. Spatial omics, consisting of spatial transcriptomics and proteomics through techniques like Visium, CODEX and MIBI-TOF, add the spatial dimension. Spatial omics allows for gene expression and protein localization directly on tissue slides and therefore can help determine the spatial organization of cellular interactions and functional niches, such as immune-excluded and inflammatory regions. The use of all these technologies together would lead to the transition of tumors from molecular slurry to cellular cartography, and new cellular targets and biomarkers can be developed according to their ecosystemic contexts [67].

6.1.2 Digital Pathology and AI-Driven Integrative Analysis

The use of digital pathology combined with AI-based integration of analysis has been noted to emerge as a potential technology in the characterization of tumor heterogeneity. Morphologic features that are hard to discern through microscopic assessment can be identified via advanced computational methods such as deep learning, from digitized images obtained from whole-slide histopathology scans. These extracted features can then be correlated with changes occurring at the molecular and epigenetic levels of the cells through high-resolution profiling techniques, which include single-cell sequencing approaches for studying epigenetic diversity in tumors at the cell level. The advent of single-cell sequencing has greatly improved the study of tumor heterogeneity since it has enabled comprehensive epigenetic profiling of single tumor cells, thus complementing histopathological findings [68].

6.2 Advanced Analytics and Trial Design

The vast volume of data calls for novel ways of analysis and improved clinical research approaches.

6.2.1 Artificial Intelligence for Predictive Modeling and Drug Discovery

Machine learning (ML) and graph neural networks applied to understand complex datasets are increasingly becoming indispensable in AI. AI has the ability to combine a multi-omic profile of a patient with real-world outcomes data to determine the best sequences of therapy to use, find synthetic lethal interactions to combine therapy, and predict the likelihood of certain adverse events. In drug discovery, AI can be used to speed up the process of identifying a target and finding novel drug candidates by processing genetic dependency screens (e.g., CRISPR data) and modeling molecular structures and their interactions. Such methods are transforming the target-first, serendipitous drug development paradigm into the patient-first, systems-driven approach [69].

6.2.2 Real-World Evidence (RWE) Generation and Adaptive "Smart" Clinical Trials

Randomized Controlled Trials (RCTs) are not only slow and costly but also do not include normal patients. Regulators are now considering RWE (based on EHRs, genomic datasets, and patient registries) to complement drug label expansion and the investigation of long-term effectiveness. At a similar time, the design of clinical trials is changing. Adaptive platform trials (such as the NCI-MATCH or industry-sponsored master protocols) integrate multiple targeted therapies in one, flexible infrastructure, testing them against multiple biomarkers at the same time. Basket trials (single drug, several types of cancers sharing a common biomarker) and umbrella trials (a single type of cancer, many drugs assigned by biomarker) are by nature effective. Smart trials employ pre-set algorithms to modify, involving closing of unsuccessful arms, opening of new ones and re-allocation of patients on-the-fly, depending on the emerging information, achieving a remarkably fast rate of therapeutic validation [70].

6.2.3 Systems Biology: Integrating Multi-Omic Data for Holistic Patient Profiles

The final aim is to have a single quantitative representation of a person's cancer. Systems biology uses both computational and mathematical modelling to combine genomic, transcriptomic, proteomic, and metabolomic data into a unified network. This method goes beyond listing changes to their dynamical interactions and systems-level behaviour. It can emulate the effect of perturbing one of the nodes (with a drug) on the whole network in a manner that predicts both resistance and the efficacy mechanisms. An up-and-coming area in the real sense of personalized treatment simulation and selection comes with the development of computational digital twins of a disease in a patient, virtual, dynamic models that can be predicted in silico to different therapies [71]. The importance of network pharmacology as a conceptual framework in precision oncology arises due to the complexity of tumours as biomolecular networks of interactions rather than just genomic alterations. For instance, a variety of cancer pathways like PI3K/AKT/mTOR, MAPK, JAK/STAT, and WNT/β-catenin have demonstrated extensive cross-talks and compensations amongst each other, hence rendering monotherapeutic interventions ineffective. On these lines, innovative computational approaches can leverage information provided by studies on transcriptomics, proteomics, phosphoproteomics, and protein-protein interactions to aid in determining key signaling nodes and synthetically lethal pathways. Combinatorial treatment can then be designed for inhibiting these complementary cancer pathways to prevent therapeutic resistance. There have been various instances of such therapies, such as dual EGFR/MET therapy for resistant NSCLC, dual BRAF/MEK therapy for melanomas, and combinatorial use of PARP inhibitors alongside immune checkpoint inhibitors in cancers deficient in homologous recombination. Hence, network pharmacology heralds a new age of precision oncology [72].

6.3 Toward Preventive and Dynamic Care

The course of action for precision oncology is early intervention and ongoing, evidence-based management, which will transform cancer into a chronic condition to be managed.

6.3.1 Early Detection and Cancer Interception via Liquid Biopsy

The use of liquid biopsy is undergoing a change towards the management of metastatic disease to detecting cancer at an early stage and screening. Multi-cancer early detection (MCED) tests are tests based on the analysis of plasma ctDNA methylation patterns, fragmentomes, and protein biomarkers to identify and predict the tissue of origin of a cancer. Although it is still debated on false positives, cost and clinical usefulness of diagnosing the very early-stage disease, these tests promise a paradigm of cancer interception. A positive signal has the potential of causing sheltered imaging and eventually, leading to curative indective or localized intervention within the stage of symptomatic manifestation. This changes the approach of managing the advanced disease to preventing the disease [73].

6.3.2 Longitudinal Monitoring for Adaptive Therapy

Treatment must focus on adaptive therapy, which is an ecological concept in which the pressure of treatment is regulated dynamically to ensure that the populated tumor remains stable and sensitive and resistant clones are suppressed. It is facilitated by ultra-sensitive longitudinal ctDNA monitoring, which offers a continuous, quantitative report on tumor burden. Rather than prolonged or maximum time or dose therapy up to progression, the therapy (dose, agent, or combination) may be adjusted according to the molecular response in real-time. This is a long-term objective of tumor control with minimum toxicity, shifting cancer treatment into a sequence of independent, reactive courses of therapy to an active, chronic disease management system driven by an unending flow of molecular information [74].

7. Conclusion

Precision oncology through comprehensive molecular profiling has redefined both the scientific and clinical landscapes of cancer care. The advances in high throughput next generation sequencing (NGS), multi-omics integration, and liquid biopsy techniques have made possible systematic detection of actionable alterations as well as real-time, minimally invasive monitoring of disease. These developments have paved way for a paradigm shift toward histology-agnostic strategies in which treatment decisions are not necessarily based on the site from which tumor originated, but also on other molecular characteristics of it. Now, clinical implementation is bolstered by established methods of analysis, databases and interdisciplinary tumor boards for molecular interpretation.

The major task facing precision oncology is not demonstrating feasibility anymore, but rather making it

available in a judicious, effective and equitable way. It should be successful through improved overall survival and quality of life as well as reduced morbidity due to appropriate choice of therapy and elimination of excessive toxicities. To accomplish this goal, there should be synergistic efforts from different stakeholders including clinicians, technologists and patients.

It is crucial to conduct global efforts in sharing information and working together in order to validate biomarkers and produce evidence. This is illustrated by AACR Project GENIE initiative. Standardization of testing approaches, bioinformatics analysis pipelines, criteria for variant interpretation, and report format is crucial in ensuring analytical validity and compatibility. There is need to invest in training of molecular pathologists, bioinformaticians, and clinicians who understand the field of genomics in parallel with these considerations. Most importantly, the issue of equitable uptake necessitates flexible reimbursement models, cost-effective and contextually appropriate test paradigms, development of facilities in underserved regions, and diversified genomic databases. Indeed, precision oncology is a constantly evolving revolution that shall realize its full potential in medicine only by adhering to the principles of rigorous science, collaboration, and universal benefit.

Authors Contributions

Imran Khan Yousafzai served as the manuscript's corresponding author, overseeing the drafting and coordination. Khadija Tariq and Noor Fatima assisted with the literature review and authoring of sections on molecular profiling technology and clinical translation. Nadia Noreen helped to write the sections on implementation issues and socioeconomic constraints. Eisha Rehman Kayani, Zain Ul Abidin, and Aqsa Mehreen contributed to the literature review on immunotherapy biomarkers, resistance mechanisms, and upcoming technologies, as well as the final manuscript preparation and revisions. All authors evaluated and approved the final manuscript for submission.

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Authors declare no conflict of interest.

Generative AI Statement

The authors certify that no artificial intelligence generation technology was employed in the preparation of this manuscript.

List of Abbreviations

Abbreviation	Full Form	Abbreviation	Full Form
AACR	American Association for Cancer Research	MIBI-TOF	Multiplexed Ion Beam Imaging by Time-of-Flight
ADC	Antibody-Drug Conjugate	ML	Machine Learning
AI	Artificial Intelligence	MLH1	MutL Homolog 1
AKT	Protein Kinase B (also known as PKB)	MRD	Minimal Residual Disease
ALK	Anaplastic Lymphoma Kinase	MRI	Magnetic Resonance Imaging
AMP	Association for Molecular Pathology	MS	Mass Spectrometry
ASCO	American Society of Clinical Oncology	MSI	Microsatellite Instability
BCR-ABL1	Breakpoint Cluster Region - Abelson Murine Leukemia Viral Oncogene Homolog 1	MSI-H	Microsatellite Instability-High
BRAF	B-Rapidly Accelerated Fibrosarcoma (proto-oncogene)	MTB	Molecular Tumor Board
BRCA1/2	Breast Cancer Gene 1 and 2	mAb	Monoclonal Antibody
CAP	College of American Pathologists	mIHC/IF	Multiplex Immunohistochemistry/Immunofluorescence
CDS	Clinical Decision Support	NCI	National Cancer Institute
CDx	Companion Diagnostic	NGS	Next-Generation Sequencing
CGP	Comprehensive Genomic Profiling	NMR	Nuclear Magnetic Resonance
CHIP	Clonal Hematopoiesis of Indeterminate Potential	NSCLC	Non-Small Cell Lung Cancer
CIBERSORT	Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts	NTRK	Neurotrophic Tyrosine Receptor Kinase

Abbreviation	Full Form	Abbreviation	Full Form
CML	Chronic Myeloid Leukemia	ORR	Objective Response Rate
CODEX	Co-Detection by Indexing	PARP	Poly (ADP-Ribose) Polymerase
CRC	Colorectal Cancer	PCR	Polymerase Chain Reaction
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats	PD-1	Programmed Cell Death Protein 1
CT	Computed Tomography	PD-L1	Programmed Death-Ligand 1
ctDNA	Circulating Tumor DNA	PET	Positron Emission Tomography
CTLA-4	Cytotoxic T-Lymphocyte Associated Protein 4	PI3K	Phosphoinositide 3-Kinase
CTC	Circulating Tumor Cell	PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
CUP	Cancer of Unknown Primary	POLE	DNA Polymerase Epsilon
dMMR	Mismatch Repair Deficiency	PROTAC	Proteolysis-Targeting Chimera
DNA	Deoxyribonucleic Acid	PTEN	Phosphatase and Tensin Homolog
EGFR	Epidermal Growth Factor Receptor	RCT	Randomized Controlled Trial
EHR	Electronic Health Record	RNA	Ribonucleic Acid
EMA	European Medicines Agency	RNA-seq	RNA Sequencing
EMT	Epithelial-Mesenchymal Transition	RWE	Real-World Evidence
EML4-ALK	Echinoderm Microtubule-Associated Protein-Like 4 - Anaplastic Lymphoma Kinase	scATAC-seq	Single-Cell Assay for Transposase-Accessible Chromatin Sequencing
ER	Estrogen Receptor	scRNA-seq	Single-Cell RNA Sequencing
EV	Extracellular Vesicle	STING	Stimulator of Interferon Genes
FDA	Food and Drug Administration	TAT	Turnaround Time
FISH	Fluorescence In Situ Hybridization	TCR	T-Cell Receptor
GENIE	Genomics Evidence Neoplasia Information Exchange	TCGA	The Cancer Genome Atlas
GINA	Genetic Information Nondiscrimination Act	TGF-b	Transforming Growth Factor Beta
HER2	Human Epidermal Growth Factor Receptor 2	TIGIT	T Cell Immunoreceptor with Ig and ITIM Domains
HR	Hormone Receptor	TIL	Tumor-Infiltrating Lymphocyte
HRD	Homologous Recombination Deficiency	TIM-3	T-Cell Immunoglobulin and Mucin Domain-Containing Protein 3
HTA	Health Technology Assessment	TKI	Tyrosine Kinase Inhibitor
ICI	Immune Checkpoint Inhibitor	TMB	Tumor Mutational Burden
IDH	Isocitrate Dehydrogenase	TMB-H	Tumor Mutational Burden-High
IFN-y	Interferon Gamma	TME	Tumor Microenvironment
IHC	Immunohistochemistry	TP53	Tumor Protein 53
IL-10	Interleukin-10	TROP-2	Trophoblast Cell Surface Antigen 2
JAK/STAT	Janus Kinase / Signal Transducer and Activator of Transcription	TRK	Tropomyosin Receptor Kinase
KRAS	Kirsten Rat Sarcoma Viral Proto-Oncogene	VEGF	Vascular Endothelial Growth Factor
LAG-3	Lymphocyte Activation Gene-3	VUS	Variants of Uncertain Significance
LMIC	Low- and Middle-Income Country	WES	Whole-Exome Sequencing

Abbreviation	Full Form	Abbreviation	Full Form
MAPK	Mitogen-Activated Protein Kinase	WGS	Whole-Genome Sequencing
MCED	Multi-Cancer Early Detection	WHO	World Health Organization
MEK	Mitogen-Activated Protein Kinase Kinase	WNT/b-catenin	Wingless-Type MMTV Integration Site / Beta-Catenin Signaling Pathway
MET	Mesenchymal-Epithelial Transition Factor (proto-oncogene)	YAP/TAZ	Yes-Associated Protein / Transcriptional Coactivator with PDZ-Binding Motif
MHC	Major Histocompatibility Complex		

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