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Challenges and Frontiers of Targeted Gene Panels in Precision Oncology.

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ABSTRACT:

Targeted gene panels (TGPs) have redefined precision oncology by bridging the gap between genomic insights and personalized treatments. Their ability to detect actionable mutations, optimize therapeutic strategies, and provide robust tumor profiling underscores their pivotal role in modern oncology. However, the rapid evolution of genomic knowledge demands continuous innovation in panel design, bioinformatics, and clinical integration. Addressing limitations such as standardization and accessibility are critical for maximizing their global impact. The integration of artificial intelligence, enhanced clinical decision support systems, and real-time updates from genomic databases promises a future where TGPs drive more equitable, precise, and effective cancer care. Through collaborative efforts across scientific, clinical, and technological domains, TGPs will continue to shape the landscape of precision medicine, ensuring better outcomes for diverse patient populations. This review delves into the pivotal role of targeted gene panels (TGPs) in cancer genomics, exploring their methodologies for target enrichment, diverse applications, and the challenges they face in clinical implementation. As this review navigates the intricacies of TGPs, it will also highlight future directions that promise to enhance their utility in genetically guided precision oncology, aiming to improve patient outcomes through personalized treatment approaches.

Keywords: Genomic profiling- Enrichment approaches- Polygenic risk scores- Tumor mutation burden- Clinical decision support systems

INTRODUCTION

In the evolving landscape of precision oncology, targeted gene panels (TGPs) have revolutionized the diagnosis, treatment, and monitoring of cancer. By focusing on specific genetic mutations associated with various cancers, these panels enhance diagnostic precision, guide personalized treatments, and improve overall patient outcomes. This personalized approach minimizes side effects while maximizing therapeutic efficacy, marking a shift from one-size-fits-all treatment models to highly customized care plans (Durães et al., 2022). Targeted gene panels, powered by next-generation sequencing (NGS), are essential tools for analyzing disease- or phenotype-specific mutations. These panels offer focused insights into genetic variations, ranging from small hotspot panels covering 1 to 50 genes to large panels that target hundreds of genes, including non-coding regions. A comparison between small and large panels is shown in Table 1. This technological flexibility enables TGPs to fulfill diverse clinical and research needs, thereby enhancing diagnostic accuracy and facilitating tailored treatment strategies based on patient-specific profiles (Anaclerio et al., 2023).

TGPs facilitate detailed genetic profiling, enabling the identification of hereditary and tumor-specific mutations. By analyzing multiple genes simultaneously, these panels improve the sensitivity and specificity of detecting cancer-related genetic alterations. This comprehensive approach aids in early cancer detection and risk stratification. Additionally, their applications in research and genetic counseling enhance our understanding of cancer biology and guide risk management strategies for patients and their families (McCabe et al., 2019).

Beyond diagnosis and treatment guidance, TGPs play a critical role in precision cancer medicine by identifying actionable mutations associated with therapeutic interventions. The integration of advanced technologies, such as liquid biopsies and RNA sequencing, enhances their utility by enabling the monitoring of disease progression and the detection of treatment resistance. As technology advances, the role of TGPs is expected to expand further, driving advancements in personalized treatment strategies tailored to individual genetic profiles and improving patient outcomes (Nagahashi et al., 2018).

Building on this overview of TGPs, the following sections will analyze their methodological framework, explore various clinical and research applications, and address key challenges and limitations. Finally, this review will discuss future directions and the evolving role of TGPs in advancing precision oncology.

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Table 1
Key Differences Between Small and Large Targeted Gene Panels

Feature	Small Targeted Gene Panels	Large Targeted Gene Panels
Size	Typically contain fewer than 50 genes	Comprise hundreds of genes
Focus	Concentrate on specific cancer hotspot genes or driver mutations	Provide a comprehensive assessment of the mutational landscape
Diagnostic Utility	Effective for identifying clinically actionable variants, but may miss some mutations	Capable of detecting a wider array of variants, including complex biomarkers
Turnaround Time	Generally faster due to simpler data analysis	Longer processing times due to complexity
Cost	More cost-effective and suitable for routine testing	Higher costs associated with broader coverage
Data Interpretation	Easier to manage with fewer variants of uncertain significance (VUS)	Larger datasets may include numerous VUS, complicating interpretation
Clinical Application	Often used for specific indications like lung cancer screening	More suited for comprehensive genomic profiling and advanced therapeutic decision-making
Sensitivity and Specificity	High sensitivity and specificity for included genes	Higher overall variant detection but may not impact patient management significantly beyond medium-sized panels

Note. Adapted from Vail et al. (2020).

1. Different Technologies for Targeted Multigene Panel Detection in Cancer

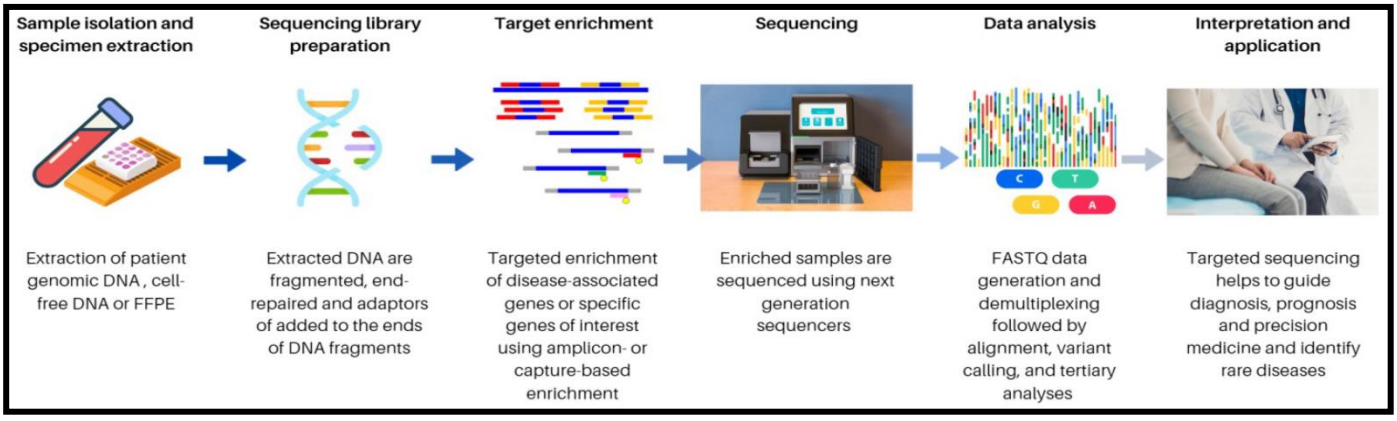
While NGS is the most common and versatile technology for analyzing targeted gene panels, other molecular techniques are also employed depending on the clinical context, cost, infrastructure, and the type of genetic information needed. The following are key technologies used for multigene panel testing:

1.1 Targeted Sequencing

Targeted sequencing, also known as panel sequencing, is an NGS approach that focuses on detection of a specific set of frequently mutated genes in cancer, enabling the simultaneous analysis of multiple genomic regions. Panels can range from as few as two genes to over 1,000, depending on their design and purpose. TGPs can be either predefined or custom-designed. During DNA preparation, target regions are enriched using hybridization capture or polymerase chain reaction (PCR) amplification, ensuring that only the gene panel of interest is sequenced, while other genomic regions remain untested (Durães et al., 2022).

Figure 1 illustrates the targeted sequencing workflow, which involves nucleic acid isolation, library preparation, and target enrichment using either amplicon-based PCR or hybridization capture methods. This process focuses sequencing on specific genomic regions.

Figure 1
Targeted Sequencing Workflow



Note. Adapted from Pei et al. (2023).

These TGPs detect single nucleotide variants (SNVs or point mutations), small insertions and deletions (indels), copy number alterations (CNAs), structural variants (SVs), and gene fusions. They can be customized to target hotspot regions within specific genes (e.g., exons 9 and 20 of *PIK3CA*, exon 15 of *BRAF*, or exons 18–21 of *EGFR*) or to encompass entire coding and noncoding sequences of genes, such as *KRAS*, *NRAS*, or *TP53* (Pei et al., 2023).

The design, content, and size of TGPs vary depending on their clinical or research applications. Smaller panels tend to focus on key driver mutations or hotspot genes relevant to specific cancers, providing rapid and cost-effective diagnostic insights.

In contrast, larger panels cover a broader mutational landscape, supporting pan-cancer screening, tumor mutation burden (TMB) assessment, and comprehensive genomic profiling. These larger panels are particularly valuable for understanding complex tumor biology and guiding therapeutic decision-making (Satam et al., 2023).

1.1.1 Targeted Enrichment approaches

There are two main approaches for target enrichment of genomic regions, as shown in Figure 2, with a comparison between these approaches presented in Table 2.

I. Amplicon-based Enrichment (Amplicon Sequencing)

Amplicon sequencing utilizes PCR to selectively amplify specific genomic regions before library preparation. This method employs specifically designed primers that target regions of interest, ensuring high sensitivity and specificity for mutation detection (Singh, 2022).

Advantages:

- Cost-Effective: Amplicon-based methods often require less starting material and are more affordable than hybridization capture methods.
- High On-Target Reads: This approach generates a higher number of on-target reads, making it suitable for applications with limited DNA availability.
- Simplified Workflow: The process involves fewer steps compared to hybridization capture, streamlining the overall procedure.

Limitations:

- Limited Target Size: Amplicon sequencing is most effective for smaller target regions, usually covering fewer than 50 genes, which restricts its use in comprehensive genomic analyses.
- PCR Bias: The reliance on PCR can introduce biases, particularly in regions with high GC content or complex genomic structures.

II. Hybridization Capture

Hybridization capture is a method that involves fragmenting DNA and enriching targeted regions using biotinylated oligonucleotide probes to selectively capture the regions of interest. This technique enables a broad analysis of large genomic regions (Singh, 2022).

Advantages:

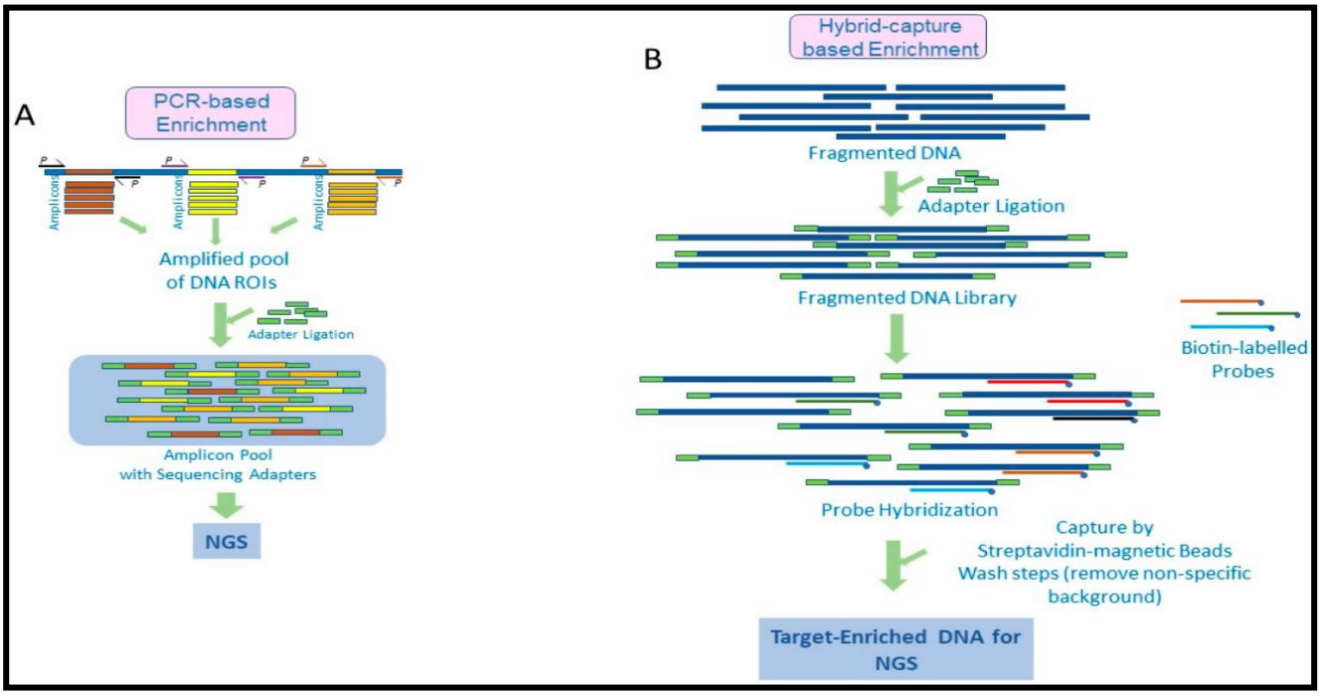
- Uniform Coverage: Hybridization capture ensures more uniform coverage across targeted regions, which is advantageous for detecting low-frequency variants. This is particularly useful for challenging specimens, such as formalin-fixed paraffin-embedded (FFPE) tissues and circulating tumor DNA (ctDNA), where PCR artifacts are more common.
- Large Target Panels: This method can accommodate an extensive number of targets, making it ideal for comprehensive genomic analysis.
- Reduced PCR Duplicates: The random shearing of DNA reduces the likelihood of identical amplicons aligning to the same genomic coordinates, minimizing computational artifacts.

Limitations:

- Higher Cost and Complexity: Hybridization capture is more expensive and involves a more complex workflow compared to amplicon-based methods.
- Longer Processing Time: The additional steps required for hybridization extend the overall workflow duration, leading to a longer turnaround time.

Figure 2

Target Enrichment Approaches for NGS



Note. (A) The PCR amplicon-based method; (B) The hybrid capture method. Adapted from Singh (2022).

Table 2
Comparison of Amplicon-Based and Hybrid Capture-Based Target Enrichment

Category	Hybrid Capture-Based Enrichment	PCR Amplicon-Based Enrichment
Enrichment Principle	Hybridization capture-based enrichment using single-stranded DNA or RNA probes complementary to the genomic regions of interest	PCR-based amplification using sequence-specific primers flanking genomic regions of interest
Nucleic Acid Input	Requires relatively high quantity of nucleic acid input	Compatible with low quantity of nucleic acid input
Nucleic Acid Quality	Compatible with challenging sample types; however, success depends on obtaining sufficient yield	Compatible with challenging sample types (e.g., FFPE and decalcified samples)
Fragmentation of Nucleic Acid Input	Required. Nucleic acids need to be enzymatically digested or acoustically sheared prior to hybrid capture	Not required
Workflow Time	Relatively long due to the hybrid capture step	Significantly shorter workflow
Workflow Complexity	High complexity workflow with multiple steps	Relatively simple workflow
Gene Targets per Panel	No limitations and can include any number of gene targets. Preferred methodology for large panels and whole-exome sequencing	Generally suited for a smaller number of gene targets. Limited by the multiplexing capability of the primers
Uniformity of Sequence Enrichment	Higher uniformity of target enrichment and lower rates of sequencing failures in regions of interest	Relatively low target enrichment uniformity and higher sequencing failures
Off-Target Sequencing Rate	Relatively high. Has more possibility of off-target sequences captured and sequenced	Lower off-target sequencing rate
Commercial Options	SureSelect (Agilent Technologies), Haloplex (Agilent Technologies), XGen NGS Hybrid Capture (Integrated DNA Technologies), TruSight Hybrid Capture (Illumina Inc.), Swift Hybrid Capture (Swift Biosciences, Ann Arbor, MI, USA)	AmpliSeq (ThermoFisher Scientific), AccesArray (Fluidigm Corporation, South San Francisco, CA, USA), GeneRead (Qiagen), RainStorm (RainDance Technologies, Lexington, MA, USA), TruSeq (Illumina Inc.), HEAT-Seq (Roche), XGen NGS Amplicon Sequencing (Integrated DNA Technologies), Accel-Amplicon (Swift Biosciences)

Note. Adapted from Singh (2022).

1.1.2 Advantages of targeted sequencing compared to other NGS technologies

In the realm of genomic analysis, targeted sequencing provides a focused and efficient alternative to whole-genome sequencing (WGS) and whole-exome sequencing (WES). By concentrating on specific genes or regions of interest, it minimizes the generation of extraneous data, particularly variants of uncertain significance (VUS), thereby reducing both the complexity of data interpretation and associated costs. This approach is especially beneficial in clinical settings, where rapid and precise identification of actionable mutations is crucial for guiding personalized treatments (Clabout et al., 2022).

1.1.3 Validation for NGS Oncology Panels

The validation of TGPs across diverse cancer types presents significant challenges that can impact their accuracy and reliability. To navigate these complexities, the Association for Molecular Pathology (AMP) and the College of American Pathologists (CAP) recommend that laboratory directors carefully evaluate key factors when selecting clinical NGS platforms, whether opting for commercially available panels or designing custom ones (Jennings et al., 2017). These factors include:

I. Clinical Indication of the Test

The selection of genes and panels should align with the test's intended application. Germline testing requires different gene targets than those used for sporadic cancers, and the analysis of solid tumors often differs from that of hematological malignancies. Pan-cancer panels are particularly advantageous as they allow for sample batching across multiple indications, resulting in cost savings, reduced labor, and shorter turnaround time.

II. Panel Size and Gene Coverage

The number of genes included, and the depth of their coverage are important considerations. Single nucleotide variants (SNVs) are the most common type of mutation in both solid tumors and hematological malignancies, such as *KRAS* p.Gly12 variants (e.g., p.Gly12Asp), *PIK3CA* p.His1047Arg, and *EGFR* p.Leu858Arg.

III. Assessment of Gene Copy Number

Copy number alterations (CNAs), which involve structural changes resulting in gains or losses of chromosomal regions, are common in solid tumors and can affect both tumor suppressor genes and oncogenes. For example, mutations in *TP53*, one of the most frequently mutated genes in cancer, are often accompanied by loss of the remaining wild-type allele. Conversely, copy number gains, such as amplifications of *ERBB2 (HER2)* in breast and gastric cancers, also hold significant clinical relevance.

IV. Additional Considerations

Other factors influencing panel selection include the expected testing volume, required turnaround time, extent of technical and bioinformatics support provided by the manufacturer, technological innovation, platform flexibility, and the laboratory's technical expertise and available resources.

Robust quality control measures are essential for ensuring the reliability of targeted gene panels. Continuous monitoring throughout the analytical process helps detect potential errors, such as allele dropout due to PCR primer mismatches or challenges in sequencing GC-rich regions. Additionally, an optimized bioinformatics pipeline is crucial for accuracy, as variant-calling algorithms must be rigorously refined to minimize variability and improve result consistency and reproducibility (Jennings et al., 2017).

1.1.4 Efforts Towards Standardizing Somatic Variant Classification and Clinical Relevance

A joint consensus from the AMP, American Society of Clinical Oncology (ASCO), and CAP proposed a guideline for categorizing somatic variants based on their clinical significance (Li et al., 2016).

Somatic variants, including SNVs, indels, fusion genes, and CNVs, differ from germline variants because their interpretation focuses on clinical impact rather than disease causality.

A variant is considered clinically relevant if it:

- Predicts therapy response, resistance, or toxicity
- Alters gene function, making it targetable by approved or investigational drugs
- Serves as a clinical trial inclusion criterion
- Influences disease prognosis
- Aids in cancer diagnosis
- Supports early detection and surveillance strategies

To standardize interpretation, this guideline classifies somatic variants into four levels based on the strength of available evidence. Table 3 summarizes these categories, while Figure 3 visually represents the different tiers. This classification system supports an evidence-based approach to variant interpretation, which helps clinicians integrate molecular findings into cancer diagnosis, prognosis, and treatment decisions.

1. Level A: Biomarkers with FDA-approved predictive significance or inclusion in professional guidelines as therapeutic, diagnostic, or prognostic markers for a specific cancer type.
2. Level B: Biomarkers supported by well-powered studies and expert consensus, predicting therapy response or resistance, or having diagnostic/prognostic value.
3. Level C: Biomarkers associated with off-label use of FDA-approved therapies or used as inclusion criteria for clinical trials, with diagnostic/prognostic significance based on multiple small studies.
4. Level D: Biomarkers with potential therapeutic relevance from preclinical studies or small-scale reports, lacking expert consensus but suggesting possible diagnostic or prognostic utility.

Table 3

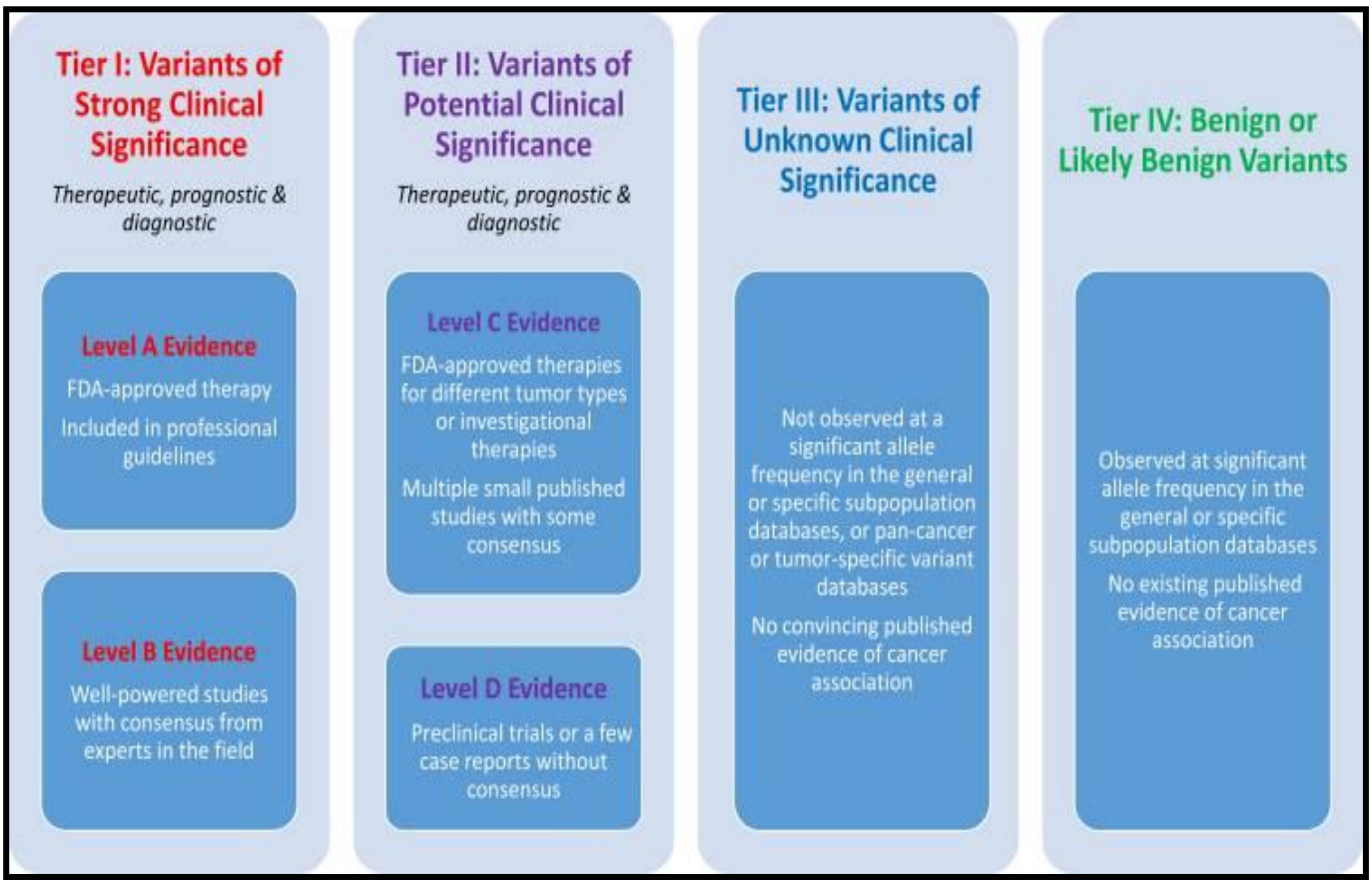
Categories of Clinical and/or Experimental Evidence for Somatic Variant Classification

Category	Therapeutic	Diagnosis	Prognosis
Level A	1. Biomarkers that predict response or resistance to FDA-approved therapies for a specific type of tumor. 2. Biomarkers included in professional guidelines that predict response or resistance to therapies for a specific type of tumor	Biomarkers included in professional guidelines as diagnostic for a specific type of tumor	Biomarkers included in professional guidelines as prognostic for a specific type of tumor
Level B	Biomarkers that predict response or resistance to therapies for a specific type of tumor based on well-powered studies with consensus from experts in the field	Biomarkers of diagnostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field	Biomarkers of prognostic significance for a specific type of tumor based on well-powered studies with consensus from experts in the field
Level C	1. Biomarkers that predict response or resistance to therapies approved by the FDA or professional societies for a different type of tumor 2. Biomarkers that serve as inclusion criteria for clinical trials	Biomarkers of diagnostic significance based on the results of multiple small studies	Biomarkers of prognostic significance based on the results of multiple small studies

Category	Therapeutic	Diagnosis	Prognosis
Level D	Biomarkers that show plausible therapeutic significance based on preclinical studies	Biomarkers that may assist disease diagnosis themselves or along with other biomarkers based on small studies or a few case reports	Biomarkers that may assist disease prognosis themselves or along with other biomarkers based on small studies or a few case reports

Note. FDA stands for Food and Drug Administration. Adapted from Li et al. (2016).

Figure 3
Evidence-Based Categorization of Somatic Variants



Note. Somatic variants are classified into four tiers based on their level of clinical significance in cancer diagnosis, prognosis, and/or therapeutics. Adapted from Li et al. (2016).

1.1.5 From Evidence to Action: How the European Society for Medical Oncology (ESMO) ESCAT Enhances Targeted Gene Panel Utility

The ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) is a transformative ranking system that enhances the utility of TGPs in oncology. It provides a structured framework for classifying, assessing, and prioritizing the clinical relevance of genomic alterations. This systematic approach facilitates the integration of NGS data into clinical decision-making, ultimately improving patient care and treatment outcomes (Mateo et al., 2018). By 2021, ESCAT rankings were formally incorporated into ESMO treatment recommendations for gastrointestinal stromal tumors and metastatic breast cancer (Casali et al., 2021; Gennari et al., 2021). Ongoing refinements since 2018 have transformed ESCAT into both a clinical decision-making tool and a reimbursement benchmark, with current applications spanning more than 15 cancer types and guiding molecular tumor boards worldwide (Mosele et al., 2024).

1.2 Alternative Technologies for Targeted Gene Panel Detection: Beyond Next-Generation Sequencing

1.2.1 Real-Time Polymerase Chain Reaction (qPCR):

This technology allows for the amplification and quantification of specific DNA sequences, making it suitable for detecting known mutations in targeted gene panels. qPCR is particularly effective for analyzing a limited number of genes or specific mutations within those genes. Multiplex PCR enables the simultaneous amplification of multiple genetic regions in a single reaction. This method is often combined with other technologies for targeted mutation

analysis or used as a standalone approach for targeted gene panels.

- **Applications**: The AmoyDx Lung Cancer PCR Panel is a commercial qPCR panel used for detecting mutations in key oncogenes, including *EGFR, KRAS*, and *BRAF* in non-small cell lung cancer (NSCLC) patients (Sakaguchi et al., 2024).
- **Strengths**: Simplicity and Speed: it simplifies the amplification of multiple targets and is cost-effective for detecting a predefined set of mutations.
- **Limitations**: Restricted Scope: Detects only known mutations, making it unsuitable for comprehensive panels. Amplification Bias: Potential variations in amplification efficiency across targets.
  Detection limit: May not effectively detect low-frequency mutations.
  Inability to perform comprehensive genomic profiling.

1.2.2 Microarrays

- Microarrays detect large-scale CNVs or specific mutations by hybridizing DNA to pre-designed probes.
- **Applications**: The OncoScan CNV Assay is a whole-genome, microarray-based assay that enables the detection of clinically relevant copy number variations (CNVs), including copy number gains, losses, as well as loss of heterozygosity (LOH).
- **Strengths**: Comprehensive coverage and high-resolution copy number detection in priority cancer genes.
- **Limitations**: Microarrays cannot detect balanced translocations or inversions. Additionally, small copy number changes, such as exon deletions and duplications within a gene, may go undetected.

1.2.3 MassARRAY System: This technology utilizes matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to perform highly multiplexed genotyping assays. The UltraSEEK Lung Panel on the MassARRAY System is designed to detect tumor-derived driver mutations in ccfDNA from NSCLC patients (Van Der Leest et al., 2023).

2.Applications of TGPs in Cancer Genomics

TGPs are essential tools in cancer genomics. The following section explores their primary applications, supported by recent research and clinical findings.

2.1 Mutation Detection and Profiling

TGPs are designed to assess the mutation status of genes frequently implicated in cancer, focusing on specific regions to identify point mutations, insertions, deletions, CNVs, and translocations that may be missed by traditional sequencing methods. Table 4 provides examples of NGS-based targeted genomic panels.

A study conducted in Southern Italy evaluated a custom-designed multigene panel (44 genes) identified in recent literature as significantly associated with predisposition to breast, ovarian, colon, and prostate cancers. The study aimed to enhance the diagnostic sensitivity of molecular screening for hereditary breast cancer. The panel detected pathogenic variants in 12 patients (19%), with *MUTYH* being the most frequently altered gene, followed by *RNASEL, ATM, MSH6, MRE11A, and PALB2*. These findings highlight the pivotal role of TGPs and the need for expanded molecular testing beyond *BRCA* genes, particularly for patients with personal or familial histories suggestive of hereditary cancer predisposition (Nunziato et al., 2022).

Table 4

Examples of Targeted Genomic Panels Powered by Next Generation Sequencing Platforms

Platform	Genes Assessed	FDA Approval	Mutations
FoundationOne CDX (Foundation Medicine)	324	Yes	Copy number alterations, gene fusions, MSI, TMB, PDL-1 (IHC)
MSK IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) (Memorial Sloan Kettering)	468	Yes	Somatic single nucleotide variants, insertions, deletions, and microsatellite instability
Oncomine Dx Target Test (Thermofisher)	46	Yes	DNA single-nucleotide variants (SNVs) and deletions in 35 genes, RNA sequence variations from 21 genes (non-small cell lung cancer)
Caris Molecular Intelligence CDX (Caris Life Sciences)	592	Partial	DNA: copy number alterations, MSI, TMB; RNA: gene fusions, mRNA variants
Oncomine Comprehensive Assay (Thermofisher)	161	-	DNA sequencing: copy number alterations, gene fusions
Trusight Oncology 500 (Illumina)	523	-	DNA + RNA assay for assessment of small variants, TMB, MSI, splice variants, and fusions
FoundationOne Liquid	70	-	Plasma: DNA sequencing: copy number alterations, specific gene fusions for lung malignancies, MSI
Guardant360 (Guardant)	76	-	Plasma: DNA sequencing: copy number alterations, 6 gene fusions

Genetic Companion Devices			
Praxis Extended RAS Panel (Illumina)	2	Yes	*KRAS* and *NRAS* (colorectal cancer)
Therascreen KRAS RGQ PCR Kit (Qiagen)	1	Yes	*KRAS* (colorectal cancer)
BRACAnalysis CDX (Myriad Genetic Laboratories)	2	Yes	*BRCA1*, *BRCA2* (ovarian and breast cancers)
FoundationFocus CDX BRCA Assay (FoundationOne)	2	Yes	*BRCA1*, *BRCA2* (ovarian cancer)
Therascreen EGFR RGQ PCR Kit (Qiagen)	1	Yes	*EGFR* (non- small cell lung cancer)
COBAS EGFR Mutation Test V2 (Roche Molecular Systems)	1	Yes	*EGFR* (non- small cell lung cancer)
THXID BRAF Kit (Biomérieux)	1	Yes	*BRAF* (melanoma)
COBAS 4800 BRAF V600 Mutation Test (Roche Molecular Systems)	1	Yes	*BRAF* (melanoma)
Therascreen FGFR RGQ RT-PCR Kit (Qiagen)	1	Yes	*FGFR* (urothelial cancer)
Therascreen PIK3CA RGQ PCR Kit (Qiagen)	1	Yes	*PIK3CA*, tissue and plasma (breast cancer)
Myriad MYCHOICE CDX (Myriad Genetic Laboratories)	Combined assay	Yes	Loss of heterozygosity (LOH), telomeric-allelic imbalance (TAI), large-scale state transitions (LST) (ovarian cancer)

Note. Adapted from Colomer et al. (2020).

2.2 Liquid Biopsy Applications

In 2019, the FDA approved several liquid biopsy tests, including the Guardant360 CDx, which employs a 73-gene cell-free DNA (cfDNA) panel to guide treatment decisions for patients with non-small cell lung cancer (NSCLC) (Killock, 2018). Another notable test, CancerSEEK, detects eight common cancer types by analyzing tumor-specific mutations in cfDNA alongside eight protein biomarkers. These innovations underscore the role of liquid biopsies in optimizing patient selection for targeted therapies, representing a significant milestone in the advancement of personalized oncology (Killock, 2018). Table 5 presents examples of FDA-approved liquid biopsy tests.

Table 5

Examples of FDA-Approved Liquid Biopsy Tests

Liquid Biopsy Assay	Disease Type	Mutation	Manufacturer	Year Approved
Cobas® EGFR mutation test	NSCLC	*EGFR* mutation (exon 19 deletions, L858R mutation)	Roche Molecular Diagnostics	2016
Guardant 360 CDx	NSCLC	ctDNA test mutations of 73 genes Some examples: *EGFR* mutation (exon 19 deletions, L858R, and T790M) *ERBB2/HER2* activating mutations (SNVs and exon 20 insertions) *KRAS* G12C	Guardant Health	2022
Guardant 360 CDx (*newly approved*)	Breast cancer	*ESR1* missense mutations between codons 310-547	Guardant Health	2023
FoundationOne® Liquid CDx	Ovarian cancer	*BRCA1*, *BRCA2*	Foundation Medicine, Inc	2020
	NSCLC	*ALK* rearrangement		

	Breast Cancer	*PIK3CA*		
	mCRPC	*BRCA1, BRCA2, ATM*		
	Lung cancer	*EGFR* mutation (exon 19 deletions, L858R mutation)		
	NSCLC	*MET* (*exon 14 mutations*)		
	NSCLC	*KRAS* wild-type (absence of mutations in codons 12 and 13)		
therascreen® KRAS RGQ PCR Kit	NSCLC	*KRAS* G12C	QIAGEN	2021
InVisionFirst®-Lung	NSCLC	37 genes NSCLC	NeoGenomics, Inc	2020
Epi proColon®	CRC	*SEPT9*	Genestr.5 Berlin	2016
Oncomine™ Dx Target Test	NSCLC	*BRAF*, *FGFR* and *IDH1* mutations	Life Technologies Corporation	2022
	Cholangiocarcinoma	Chromosome abnormalities by rearrangement in *ROS1* and *RET*		
TSO500 ctDNA®	Pan-cancer	500+ genes	Illumina	2019
	Breast Cancer			
	Lung Cancer			
	Colorectal Cancer			
	Gastric Cancer			

Note. Adapted from Aquino & Pascut (2023).

2.3 Tumor Mutation Burden (TMB) Assessment

Li et al. (2023) evaluated the effectiveness of TGPs in estimating TMB compared to WES. Their findings demonstrated that targeted panels, when adjusted for panel size and gene-specific variability, can provide a cost-effective and accurate alternative for TMB assessment. The study emphasized their potential in guiding immunotherapy decisions, as high TMB levels correlate with improved responses to immune checkpoint inhibitors (ICIs). Similarly, Bradley and Cannings (2022) highlighted the utility of a targeted panel in estimating TMB in non-small cell lung cancer (NSCLC), which facilitates patient selection for immunotherapy based on mutational profiles.

2.4 Comprehensive Profiling in Rare Cancers

TGPs enable comprehensive genetic profiling, even in rare or less-studied cancers, deepening our understanding of their molecular underpinnings.

McCabe et al. (2019) developed PV2, a targeted gene panel comprising 451 cancer-associated genes, uniquely designed to cover entire genes rather than just specific exons or hotspots. This panel includes a broad range of genes linked to pituitary tumors, and was applied to patient cohorts with pituitary tumors, oral squamous cell carcinoma (OSCC), and cutaneous squamous cell carcinoma (cSCC). Beyond improving the detection of actionable mutations, PV2 demonstrated utility in liquid biopsies, underscoring its significance for patients with under-researched head and neck cancers and other malignancies.

2.5 Monitoring Treatment Response and Resistance

A retrospective analysis in NSCLC found no significant survival benefit from targeted multigene panel testing compared to single-gene testing for *EGFR* and *ALK*, with fewer than 5% of patients receiving additional targeted therapy (Presley et al., 2018). However, a key advantage of TGPs is their ability to extend beyond actionable variants in known genes, uncovering mechanisms of acquired resistance, such as the *EGFR* T790M mutation, which confers resistance to first-generation *EGFR* TKIs in NSCLC (Bollinger et al., 2017).These broader insights have facilitated the adoption of synergistic drug combinations, including the use of osimertinib for *EGFR*-mutant NSCLC (Soria et al., 2017) and the dabrafenib-trametinib combination for *BRAF*-mutant melanoma (Coit et al., 2016), which significantly improve survival and redefine the standard of care.

2.6 Genetic Counseling and Risk Assessment

2.6.1 Advancements in Multigene Panel Testing for Hereditary Breast Cancer

Recent advancements in multigene panel testing have significantly influenced genetic counseling, particularly in hereditary breast cancer (HBC). These panels are increasingly used to identify at-risk individuals and guide evidence-based interventions for cancer prevention and early detection. Genetic counseling plays a crucial role in this process, aiding patients in interpreting test results and understanding the potential implications for at-risk family members (Reid & Pal, 2020).

2.6.2 Navigating Complexities: The Role of Genetic Counseling

With the expansion of gene panel testing, genetic counseling approaches must adapt to navigate the complexities of VUS while emphasizing the importance of informed consent. Pretest genetic counseling has evolved to address these uncertainties, ensuring that patients are well-informed without feeling overwhelmed (Rainville & Rana, 2014). Post-test counseling remains essential for interpreting results, particularly when pathogenic or likely pathogenic variants are identified (Robson et al., 2015).

2.6.3 Enhancing Risk Prediction: The Promise of Polygenic Risk Scores (PRS)

Polygenic risk scores are valuable for predicting an individual's cancer risk, particularly when integrated with other risk factors to enhance the stratification of high-risk populations. This approach enables personalized adjustments to screening protocols, such as modifying the age of initiation or screening frequency for common cancers like breast, prostate, and colorectal cancer. While studies have demonstrated the cost-effectiveness of incorporating PRS into cancer screening strategies, further validation is required from ongoing trials, including WISDOM, MY-PEBS, and BARCODE (Xiang et al., 2024).

2.6.4 Integrating Genetic Insights: The Synergy of TGPs and PRS

Targeted multigene panel testing enhances PRS by assessing both high- and low-penetrance genetic variants associated with cancer risk. Integrating these genetic insights provides a more comprehensive understanding of an individual's cancer risk profile. The combined use of TGPs and PRS improves risk stratification, guiding personalized screening strategies and reinforcing cancer prevention and management efforts (Tsoulos et al., 2024).

3. Challenges and Limitations of Targeted Gene Panels

While TGPs have become valuable tools in genetic diagnostics and offer significant advantages in cancer genomics, including cost-effectiveness and rapid turnaround time, they also come with several challenges and limitations that may impact their clinical utility.

3.1 Challenges in Identifying Novel Genes

A major limitation of TGPs is their inability to identify novel causative genes, as they are limited to known genes associated with specific conditions or phenotypes. Additionally, once a gene panel is established, integrating newly discovered genes can be technically demanding.

3.2 Variants of Uncertain Significance (VUS)

One of the biggest challenges in interpreting TGP results is the identification of VUS. These are genetic variants that have not been definitively classified as either pathogenic or benign, and their occurrence increases with the number of genes tested (Grissom & Friend, 2016). A study highlighted that VUS are frequently misinterpreted, potentially leading to unnecessary follow-up tests and affecting clinical management. The ambiguity surrounding VUS can create confusion among healthcare providers and patients, complicating treatment decisions (Donohue et al., 2021).

3.3 Structural Rearrangements and Copy Number Variants

Unlike whole genome sequencing (WGS), which provides a more comprehensive genomic analysis, TGPs have limitations in detecting structural rearrangements and copy number variants.

3.4 Balancing Diagnostic Yield and Clinical Utility

The selection of panel size and content must balance diagnostic yield with clinical utility. While larger panels offer more comprehensive data, they also increase the likelihood of incidental findings that may complicate patient management without providing substantial clinical benefit. For instance, extensive panels used to profile advanced-stage cancer patients may not always yield actionable insights (Durães et al., 2022).

3.5 Bias Toward Common Mutations

Targeted gene panels primarily detect common mutations linked to specific cancers, which may overlook rare but clinically significant alterations. This bias may reduce their effectiveness in identifying all relevant genetic factors that influence tumor behavior and patient outcomes (Slavin et al., 2015).

3.6 The Need for Regular Update

The rapid advancement of genomic knowledge means that TGPs can quickly become outdated. As new disease-associated genes are continuously identified, panels developed more than two years ago may fail to detect a considerable number of clinically relevant mutations. This necessitates regular updates to gene panels, a process that is both resource-intensive and logistically challenging for laboratories (Quaio et al., 2021).

3.7 Analytical Challenges

3.7.1 Sample Quality Requirements

The effectiveness of TGPs is often influenced by the quality and quantity of the sample material. A study on a 22-gene panel reported high concordance between paired FFPE and fresh frozen tissue samples in detecting mutations in colorectal cancer (CRC), suggesting that FFPE tissues stored for less than two years can yield reliable results. However, factors such as fixation time, storage duration, and DNA quality may still affect accuracy (Gao et al., 2020).

While FFPE samples can be used when fresh frozen tissue is unavailable, fresh frozen samples should be prioritized for routine analysis. If mutation results from FFPE do not align with clinical responses to *EGFR*-targeted therapies, retesting with fresh frozen tissue may be necessary. Therefore, careful assessment of sample quality and handling is crucial for ensuring reliable results in targeted multigene panel testing.

3.7.2 PCR Pitfalls and Bias in Amplicon-Based Enrichment Method

While amplicon-based enrichment methods are valuable for targeted sequencing, they have several limitations compared to hybrid capture techniques, including:

- Bias and Inaccuracies: PCR amplification can introduce biases and artifacts, particularly in regions with high GC content or complex genomic structures. This may result in skewed variant frequency estimates and inaccuracies in mutation detection.

- Uneven Coverage: Amplicon-based methods often result in uneven sequencing coverage, as some genomic regions amplify more efficiently than others. This variability can compromise the accurate detection of mutations and copy number variations.
- Higher False-Positive Rates: The presence of PCR duplicates and sequencing artifacts can increase the false-positive rate in amplicon-based assays, complicating the distinction between true variants and sequencing errors.
- Limited Detection Capabilities: Although effective for detecting known mutations, amplicon-based methods may struggle to identify rare but clinically significant genomic alterations due to their reliance on predefined primers.
- Sensitivity to Sample Quality: The performance of amplicon-based methods is highly dependent on the quality of the starting material. Degraded DNA from FFPE samples can significantly impact accuracy.

3.8 The Necessity of Advanced Bioinformatics Solutions

The rapid advancement of genomic technologies necessitates a robust bioinformatics infrastructure to manage large datasets, support data sharing, and enable the reclassification of VUS over time. Without this infrastructure, the full potential of TGPs may not be realized (Yang et al., 2019).

3.9 Counseling and Communication Challenges

The complexity of targeted gene testing requires skilled genetic counseling to help patients interpret their results, particularly the implications of VUS and unexpected findings. Without adequate counseling, patients may misinterpret results, potentially leading to inappropriate clinical decisions (Coughlin et al., 2022).

3.10 Financial Implications and Accessibility Challenges

The cost of targeted gene panel testing remains a significant challenge, particularly in low- and middle-income countries, where affordability limits patient access. Clinicians must navigate these complexities, including variations in insurance coverage and financial constraints, when recommending testing (Coughlin et al., 2022).

3.11 Standardization issues and lack of harmonization in Laboratory Practices

Different laboratories offer a variety of TGPs for similar clinical indications, leading to inconsistencies in diagnostic outcomes. This variability poses significant challenges for clinicians, who must navigate the complexities of selecting the most appropriate panel for their patients' specific needs. The lack of standardization in panel offerings complicates clinical judgement, making it difficult to ensure reliable genetic testing and optimal care (Quaio et al., 2021).

Furthermore, the lack of harmonization in reporting systems remains a major challenge. Most multigene sequencing platforms neither prioritize genetic alterations nor follow a standardized, clinically relevant ranking system. This inconsistency in terminology and classification creates substantial barriers to the advancement of precision medicine by complicating clinical decision-making and increasing the risk of inappropriate treatments based on unverified or poorly understood genetic information (Horgan et al., 2022; Mateo et al., 2018).

4. Recommendations and Future directions

To enhance the utility and inclusivity of TGPs, several strategic improvements are essential:

4.1 Expanding Gene Coverage and Bioinformatics Integration

Broadening the scope of gene panels to include emerging biomarkers and rare but actionable mutations is essential for keeping pace with advancements in diagnostics. This expansion ensures that TGPs remain relevant and effective in identifying treatment options for a wider range of patients. Additionally, integrating artificial intelligence (AI)-driven bioinformatics tools can streamline variant classification and enhance the interpretation of complex genomic data, thereby improving both efficiency and accuracy in clinical settings.

4.2 Enhancing Standardization and Accessibility

Standardization is a critical priority for improvement. Establishing robust validation and reporting guidelines for TGPs will promote consistency across laboratories, enhancing the reliability of results. Additionally, addressing cost barriers through strategic initiatives and advocating for broader insurance coverage will improve accessibility for underserved communities. Moreover, advancing research that focuses on underrepresented ethnic groups will enhance the generalizability of findings, ensuring that TGPs effectively serve diverse patient populations.

4.3 AI Integration in Clinical Decision Support Systems for Cancer Genomics

TGPs in cancer genomics are poised for significant advancements, particularly with the adoption of clinical decision support systems (CDSS). The incorporation of AI algorithms and advanced bioinformatics tools will enhance genomic data interpretation, enabling more personalized treatment strategies. Refining variant interpretation within CDSS by integrating real-time updates from extensive genomic databases, alongside patient-specific clinical histories will generate tailored and clinically relevant recommendations.

4.4 Integrating Genomic Data into EHRs and Promoting Equity in Precision Oncology

Standardizing genomic data incorporation into electronic health records (EHRs) will be crucial for facilitating seamless access for healthcare providers and optimizing clinical workflows. Expanding clinical studies to improve representation across different ethnic and genetic backgrounds will further enhance the applicability of TGP findings and promote equity in precision oncology. Collaborative efforts among researchers, clinicians, and bioinformaticians will be essential for advancing TGP methodologies and translating advancements into improved patient outcomes.

Conclusion

TGPs are at the forefront of revolutionizing precision oncology, providing invaluable insights by identifying actionable mutations that guide treatment decisions. Looking ahead, the integration of advanced bioinformatics and AI-driven CDSS will enhance variant interpretation, streamline EHR integration, and enable more precise treatment strategies. These advancements will not only drive more personalized and effective cancer care but also transform the landscape of oncology, offering hope for improved outcomes and tailored treatments for every patient.

References

Anaclerio, F., Pilenzi, L., Dell'Elice, A., Ferrante, R., Grossi, S., Ferlito, L. M., Marinelli, C., Gildetti, S., Calabrese, G., Stuppia, L., & Antonucci, I. (2023). Clinical usefulness of NGS multi-gene panel testing in hereditary cancer analysis. *Frontiers in Genetics*, *14*. https://doi.org/10.3389/fgene.2023.1060504

Aquino, I. M. C., & Pascut, D. (2023). Liquid biopsy: New opportunities for precision medicine in hepatocellular carcinoma care. *Annals of Hepatology*, *29*(2), 101176. https://doi.org/10.1016/j.aohep.2023. 101176

Bollinger, M. K., Agnew, A. S., & Mascara, G. P. (2017). Osimertinib: A third-generation tyrosine kinase inhibitor for treatment of epidermal growth factor receptor-mutated non-small cell lung cancer with the acquired Thr790Met mutation. *Journal of Oncology Pharmacy Practice*, *24*(5), 379–388. https://doi.org/10.1177/1078155217712401

Bradley, J. R., & Cannings, T. I. (2022). Data-driven design of targeted gene panels for estimating immunotherapy biomarkers. *Communications Biology*, *5*(1). https://doi.org/10.1038/s42003-022-03098-1

Casali, P., Blay, J., Abecassis, N., Bajpai, J., Bauer, S., Biagini, R., Bielack, S., Bonvalot, S., Boukovinas, I., Bovee, J., Boye, K., Brodowicz, T., Buonadonna, A., De Álava, E., Tos, A. D., Del Muro, X., Dufresne, A., Eriksson, M., Fedenko, A., . . . Stacchiotti, S. (2021). Gastrointestinal stromal tumours: ESMO–EURACAN–GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, *33*(1), 20–33. https://doi.org/10.1016/j.annonc.2021.09.005

Clabout, T., Maes, L., Acke, F., Wuyts, W., Van Schil, K., Coucke, P., Janssens, S., & De Leenheer, E. (2022). Negative Molecular Diagnostics in Non-Syndromic Hearing Loss: What next? *Genes*, *14*(1), 105. https://doi.org/10.3390/genes14010105

Coit, D. G., Thompson, J. A., Algazi, A., Andtbacka, R., Bichakjian, C. K., Carson, W. E., Daniels, G. A., DiMaio, D., Fields, R. C., Fleming, M. D., Gastman, B., Gonzalez, R., Guild, V., Johnson, D., Joseph, R. W., Lange, J. R., Martini, M. C., Materin, M. A., Olszanski, A. J., . . . Engh, A. (2016). NCCN Guidelines Insights: Melanoma, Version 3.2016. *Journal of the National Comprehensive Cancer Network*, *14*(8), 945–958. https://doi.org/10.6004/jnccn.2016.0101

Colomer, R., Mondejar, R., Romero-Laorden, N., Alfranca, A., Sanchez-Madrid, F., & Quintela-Fandino, M. (2020). When should we order a next generation sequencing test in a patient with cancer? *EClinicalMedicine*, *25*, 100487. https://doi.org/10.1016/ j.eclinm.2020.100487

Coughlin, S. E., Heald, B., Clark, D. F., Nielsen, S. M., Hatchell, K. E., Esplin, E. D., & Katona, B. W. (2022). Multigene panel testing yields high rates of clinically actionable variants among patients with colorectal cancer. *JCO Precision Oncology*, *6*. https://doi.org/10.1200/po.22.00517

Donohue, K. E., Gooch, C., Katz, A., Wakelee, J., Slavotinek, A., & Korf, B. R. (2021). Pitfalls and challenges in genetic test interpretation: An exploration of genetic professionals experience with interpretation of results. *Clinical Genetics*, *99*(5), 638–649. https://doi.org/10.1111/cge.13917

Durães, C., Gomes, C. P., Costa, J. L., & Quagliata, L. (2022). Demystifying the discussion of sequencing panel size in oncology genetic testing. *European Medical Journal*, 68–77. https://doi.org/10.33590/emj/22c9259

Gao, X. H., Li, J., Gong, H. F., Yu, G. Y., Liu, P., Hao, L. Q., Liu, L. J., Bai, C. G., & Zhang, W. (2020). Comparison of fresh frozen tissue with Formalin-Fixed Paraffin-Embedded tissue for mutation analysis using a Multi-Gene panel in patients with colorectal cancer. *Frontiers in Oncology*, *10*. https://doi.org/10.3389/fonc. 2020.00310

Gennari, A., André, F., Barrios, C., Cortés, J., De Azambuja, E., DeMichele, A., Dent, R., Fenlon, D., Gligorov, J., Hurvitz, S., Im, S., Krug, D., Kunz, W., Loi, S., Penault-Llorca, F., Ricke, J., Robson, M., Rugo, H., Saura, C., . . . Harbeck, N. (2021). ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. *Annals of Oncology*, *32*(12), 1475–1495. https://doi.org/10.1016/j.annonc.2021.09.019

Grissom, A. A., & Friend, P. J. (2016, May 1). *Multigene panel testing for hereditary cancer risk*. https://pmc.ncbi.nlm.nih.gov/articles/PMC5679029/#A10

Horgan, D., Curigliano, G., Rieß, O., Hofman, P., Büttner, R., Conte, P., Cufer, T., Gallagher, W. M., Georges, N., Kerr, K., Penault-Llorca, F., Mastris, K., Pinto, C., Van Meerbeeck, J., Munzone, E., Thomas, M., Ujupan, S., Vainer, G. W., Velthaus, J., & André, F. (2022). Identifying the steps required to effectively implement Next-Generation sequencing in oncology at a national level in Europe. *Journal of Personalized Medicine*, *12*(1), 72. https://doi.org/10.3390/jpm12010072

Jennings, L. J., Arcila, M. E., Corless, C., Kamel-Reid, S., Lubin, I. M., Pfeifer, J., Temple-Smolkin, R. L., Voelkerding, K. V., & Nikiforova, M. N. (2017). Guidelines for Validation of Next-Generation Sequencing–Based Oncology Panels. *Journal of Molecular Diagnostics*, *19*(3), 341–365. https://doi.org/10.1016/j.jmoldx.2017.01.011

Killock, D. (2018). CancerSEEK and destroy — a blood test for early cancer detection. *Nature Reviews Clinical Oncology*, *15*(3), 133. https://doi.org/10.1038/nrclinonc.2018.21

Li, M. M., Datto, M., Duncavage, E. J., Kulkarni, S., Lindeman, N. I., Roy, S., Tsimberidou, A. M., Vnencak-Jones, C. L., Wolff, D. J., Younes, A., & Nikiforova, M. N. (2016). Standards and guidelines for the interpretation and reporting of sequence variants in cancer. *Journal of Molecular Diagnostics*, *19*(1), 4–23. https://doi.org/10.1016/j.jmoldx.2016.10.002

Li, D., Wang, D., Johann, D. J., Hong, H., & Xu, J. (2023). Assessments of tumor mutational burden estimation by targeted panel sequencing: A comprehensive simulation analysis. *Experimental Biology and Medicine*. https://doi.org/10.1177/ 15353702231211882

Mateo, J., Chakravarty, D., Dienstmann, R., Jezdic, S., Gonzalez-Perez, A., Lopez-Bigas, N., Ng, C., Bedard, P., Tortora, G., Douillard, J., Van Allen, E., Schultz, N., Swanton, C., André, F., & Pusztai, L. (2018). A framework to rank genomic alterations as targets for cancer precision medicine: the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). *Annals of Oncology*, *29*(9), 1895–1902. https://doi.org/10.1093/annonc/mdy263

McCabe, M. J., Gauthier, M. A., Chan, C., Thompson, T. J., De Sousa, S. M., Puttick, C., Grady, J. P., Gayevskiy, V., Tao, J., Ying, K., Cipponi, A., Deng, N., Swarbrick, A., Thomas, M. L., Lord, R. V., Johns, A. L., Kohonen-Corish, M., O'Toole, S. A., Clark, J., . . . Young, M. (2019). Development and validation of a targeted gene sequencing panel for application to disparate cancers. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-52000-3

Mosele, M., Westphalen, C., Stenzinger, A., Barlesi, F., Bayle, A., Bièche, I., Bonastre, J., Castro, E., Dienstmann, R., Krämer, A., Czarnecka, A., Meric-Bernstam, F., Michiels, S., Miller, R., Normanno, N., Reis-Filho, J., Remon, J., Robson, M., Rouleau, E., . . . André, F. (2024). Recommendations for the use of next-generation sequencing (NGS) for patients with advanced cancer in 2024: a report from the ESMO Precision Medicine Working Group. *Annals of Oncology*, *35*(7), 588–606. https://doi.org/10.1016/j.annonc.2024.04.005.

Nagahashi, M., Shimada, Y., Ichikawa, H., Kameyama, H., Takabe, K., Okuda, S., & Wakai, T. (2018). Next generation sequencing-based gene panel tests for the management of solid tumors. *Cancer Science*, *110*(1), 6–15. https://doi.org/10.1111/cas.13837

Nunziato, M., Di Maggio, F., Pensabene, M., Esposito, M. V., Starnone, F., De Angelis, C., Calabrese, A., D'Aiuto, M., Botti, G., De Placido, S., D'Argenio, V., & Salvatore, F. (2022). Multi-gene panel testing increases germline predisposing mutations' detection in a cohort of breast/ovarian cancer patients from Southern Italy. *Frontiers in Medicine*, *9*. https://doi.org/10.3389/fmed.2022.894358

Pei, X. M., Yeung, M. H. Y., Wong, A. N. N., Tsang, H. F., Yu, A. C. S., Yim, A. K. Y., & Wong, S. C. C. (2023). Targeted sequencing approach and its clinical applications for the molecular diagnosis of human diseases. *Cells*, *12*(3), 493. https://doi.org/10.3390/cells12030493

Presley, C. J., Tang, D., Soulos, P. R., Chiang, A. C., Longtine, J. A., Adelson, K. B., Herbst, R. S., Zhu, W., Nussbaum, N. C., Sorg, R. A., Agarwala, V., Abernethy, A. P., & Gross, C. P. (2018). Association of Broad-Based genomic sequencing with survival among patients with advanced Non–Small cell lung cancer in the community oncology setting. *JAMA*, *320*(5), 469. https://doi.org/10.1001/jama.2018.9824

Quaio, C. R. D. C., Obando, M. J. R., Perazzio, S. F., Dutra, A. P., Chung, C. H., Moreira, C. M., Filho, G. M. N., Sacramento-Bobotis, P. R., Penna, M. G., De Souza, R. R. F., Cintra, V. P., Carnavalli, J. E. P., Da Silva, R. A., Santos, M. N. P., Paixão, D., Da Rosa Baratela, W. A., Olivati, C., Spolador, G. M., Pintao, M. C., . . . Kim, C. A. (2021). Exome sequencing and targeted gene panels: a simulated comparison of diagnostic yield using data from 158 patients with rare diseases. *Genetics and Molecular Biology*, *44*(4). https://doi.org/10.1590/1678-4685-gmb-2021-0061

Rainville, I. R., & Rana, H. Q. (2014). Next-Generation Sequencing for Inherited Breast Cancer Risk: Counseling through the Complexity. *Current Oncology Reports*, *16*(3). https://doi.org/10.1007/s11912-013-0371-z

Reid, S., & Pal, T. (2020). Update on multi-gene panel testing and communication of genetic test results. *The Breast Journal*, *26*(8), 1513–1519. https://doi.org/10.1111/tbj.13971

Robson, M. E., Bradbury, A. R., Arun, B., Domchek, S. M., Ford, J. M., Hampel, H. L., Lipkin, S. M., Syngal, S., Wollins, D. S., & Lindor, N. M. (2015). American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic testing for cancer susceptibility. *Journal of Clinical Oncology*, *33*(31), 3660–3667. https://doi.org/ 10.1200/jco.2015.63.0996

Sakaguchi, T., Iketani, A., Esumi, S., Esumi, M., Suzuki, Y., Ito, K., Fujiwara, K., Nishii, Y., Katsuta, K., Yasui, H.,

Taguchi, O., & Hataji, O. (2024). The Current Achievements of Multi-Gene Panel Tests in Clinical Settings for Patients with Non-Small-Cell Lung Cancer. *Cancers*, *16*(9), 1670. https://doi.org/10.3390/cancers 16091670

Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., Thakare, R. P., Banday, S., Mishra, A. K., Das, G., & Malonia, S. K. (2023). Next-Generation Sequencing Technology: Current trends and advancements. *Biology*, *12*(7), 997. https://doi.org/ 10.3390/biology12070997

Singh, R. R. (2022). Target enrichment Approaches for Next-Generation sequencing Applications in Oncology. *Diagnostics*, *12*(7), 1539. https://doi.org/10.3390/ diagnostics12071539

Slavin, T. P., Niell-Swiller, M., Solomon, I., Nehoray, B., Rybak, C., Blazer, K. R., & Weitzel, J. N. (2015). Clinical application of multigene panels: Challenges of Next-Generation Counseling and cancer risk Management. *Frontiers in Oncology*, *5*. https://doi.org/10.3389/ fonc.2015.00208

Soria, J., Ohe, Y., Vansteenkiste, J., Reungwetwattana, T., Chewaskulyong, B., Lee, K. H., Dechaphunkul, A., Imamura, F., Nogami, N., Kurata, T., Okamoto, I., Zhou, C., Cho, B. C., Cheng, Y., Cho, E. K., Voon, P. J., Planchard, D., Su, W., Gray, J. E., . . . Ramalingam, S. S. (2017). Osimertinib in UntreatedEGFR-Mutated Advanced Non–Small-Cell lung cancer. *New England Journal of Medicine*, *378*(2), 113–125. https://doi.org/ 10.1056/nejmoa1713137

Tsoulos, N., Papadopoulou, E., Agiannitopoulos, K., Grigoriadis, D., Tsaousis, G. N., Bouzarelou, D., Gogas, H., Troupis, T., Venizelos, V., Fountzilas, E., Theochari, M., Ziogas, D. C., Giassas, S., Koumarianou, A., Christopoulou, A., Busby, G., Nasioulas, G., & Markopoulos, C. (2024). Polygenic Risk Score (PRS) Combined with NGS Panel Testing Increases Accuracy in Hereditary Breast Cancer Risk Estimation. *Diagnostics*, *14*(16), 1826. https://doi.org/10.3390/diagnostics 14161826.

Vail, E., Song, J., Xu, J., Frye, J. S., Kim, J. T., Pao, A., Schreck, R., Aguiluz, A. S., Zhang, W., Alkan, S., Mita, A., Mita, M., Figlin, R. A., Engman, D. M., & Lopategui, J. R. (2020). Comparison of large, medium, and small solid tumor gene panels for detection of clinically actionable mutations in cancer. *Targeted Oncology*, *15*(4), 523–530. https://doi.org/10.1007/s11523-020-00743-9

Van Der Leest, P., Janning, M., Rifaela, N., Azpurua, M. L. A., Kropidlowski, J., Loges, S., Lozano, N., Sartori, A., Irwin, D., Lamy, P., Hiltermann, T. J. N., Groen, H. J. M., Pantel, K., Van Kempen, L. C., Wikman, H., & Schuuring, E. (2023). Detection and monitoring of Tumor-Derived mutations in circulating tumor DNA using the UltraSEEK lung panel on the MassARRAY system in metastatic Non-Small cell lung cancer patients. *International Journal of Molecular Sciences*, *24*(17), 13390. https://doi.org/10.3390/ijms241713390

Xiang, R., Kelemen, M., Xu, Y., Harris, L. W., Parkinson, H., Inouye, M., & Lambert, S. A. (2024). Recent advances in polygenic scores: translation, equitability, methods and FAIR tools. *Genome Medicine*, *16*(1). https://doi.org/ 10.1186/s13073-024-01304-9

Yang, H., Shah, R. H., Tegay, D., & Onel, K. (2019). Precision oncology: lessons learned and challenges for the future *Cancer Management and Research*, *Volume 11*, 7525–7536. https://doi.org/10.2147 /cmar.s201326