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REVIEW ARTICLE

Interpreting and integrating genomic tests results in clinical cancer care: Overview and practical guidance

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Abstract

The last decade has seen rapid progress in the use of genomic tests, including gene panels, whole-exome sequencing, and whole-genome sequencing, in research and clinical cancer care. These advances have created expansive opportunities to characterize the molecular attributes of cancer, revealing a subset of cancer-associated aberrations called *driver mutations*. The identification of these driver mutations can unearth vulnerabilities of cancer cells to targeted therapeutics, which

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has led to the development and approval of novel diagnostics and personalized interventions in various malignancies. The applications of this modern approach, often referred to as *precision oncology* or *precision cancer medicine*, are already becoming a staple in cancer care and will expand exponentially over the coming years. Although genomic tests can lead to better outcomes by informing cancer risk, prognosis, and therapeutic selection, they remain underutilized in routine cancer care. A contributing factor is a lack of understanding of their clinical utility and the difficulty of results interpretation by the broad oncology community. Practical guidelines on how to interpret and integrate genomic information in the clinical setting, addressed to clinicians without expertise in cancer genomics, are currently limited. Building upon the genomic foundations of cancer and the concept of precision oncology, the authors have developed practical guidance to aid the interpretation of genomic test results that help inform clinical decision making for patients with cancer. They also discuss the challenges that prevent the wider implementation of precision oncology.

KEYWORDS
cancer genomics, education, germline, molecular profiling, precision oncology, somatic

INTRODUCTION

Over the last several decades, the molecular characterization of tumors has evolved from scientific use into a valuable tool in clinical cancer care. This was possible because of advances in molecular biology and DNA sequencing technologies, significantly accelerating our understanding of the molecular pathology of cancer. This led to the development of novel diagnostic and therapeutic modalities in various cancer types. These therapeutics are often targeted to specific molecular alterations that can be detected by molecular tumor profiling. The interplay between modern diagnostics and molecularly driven therapeutics is often referred to as *precision oncology* or *precision cancer medicine* and is revolutionizing the way we approach and treat cancer. The first example of successful targeted cancer treatments dates to the 1970s, when tamoxifen was approved for the treatment of estrogen receptor-positive breast cancer.[1] In 1984, the proto-oncogene *ERBB2*, which encodes the signaling receptor also known as HER2, was discovered in a particularly aggressive subtype of breast cancer and defined as a potential therapeutic target.[2] In 1998, trastuzumab was approved by the US Food and Drug Administration for the treatment of ERBB2 (HER2)-positive breast cancer. After the discovery of the Philadelphia chromosome, the development of imatinib for the treatment of BCR::ABL1-positive chronic myeloid leukemia marked the beginning of a new era in the therapy of malignant disease.[3, 4] Since these discoveries, there has been a rapid increase in the approval of targeted therapies.[5] In the last decade, there have been 115 new approvals for novel biomarker-associated cancer therapies. The increasing number of molecularly guided treatment options was paralleled by substantial advancements in *next-generation sequencing* (NGS) technologies, which have progressively entered the clinic. The ability to detect aberrations accurately and efficiently across multiple genes continues to transform cancer care. The concept of precision oncology, and personalized medicine in general, has been applied by clinicians for decades; however, the ability to test numerous genomic aberrations at once and directly target these vulnerabilities has revolutionized the field.

Today, NGS allows for the simultaneous sequencing of multiple genes, enabling identification of mutations, copy number variations, gene fusions, structural rearrangements, and biomarkers, such as *tumor mutational burden* (TMB) and *microsatellite instability* (MSI), within one single assay. For some cancer types, up-front NGS tumor testing is now recommended by both the American Society of Clinical Oncology (ASCO)[6] and the European Society for Medical Oncology (ESMO)[7] as standard of care for advanced cancers. These recommendations are based on established evidence for antitumor efficacy of genomic biomarker-linked therapies with regulatory approval of agents based on specific genomic alterations. More recently, histology (tumor)-agnostic, genomic biomarker-driven therapies have been approved for advanced cancer, further expanding the need for scalable genomic profiling in oncology.[8]

A plethora of novel biomarker-guided, targeted agents are in late-stage clinical development, not only in the metastatic setting but also in early stage disease. This will further expand the utility of genomic sequencing by identifying additional alterations for which biomarker-directed therapies are available, thus increasing the proportion of patients requiring molecular profiling for routine clinical care (Figure 1).

Although cancer genomics should be widely adopted as part of routine clinical care, certain challenges hamper its appropriate use and clinical implementation. Among the various challenges, there is a general lack of clarity regarding when molecular profiling should be performed, what type of assays should be used, and how the results

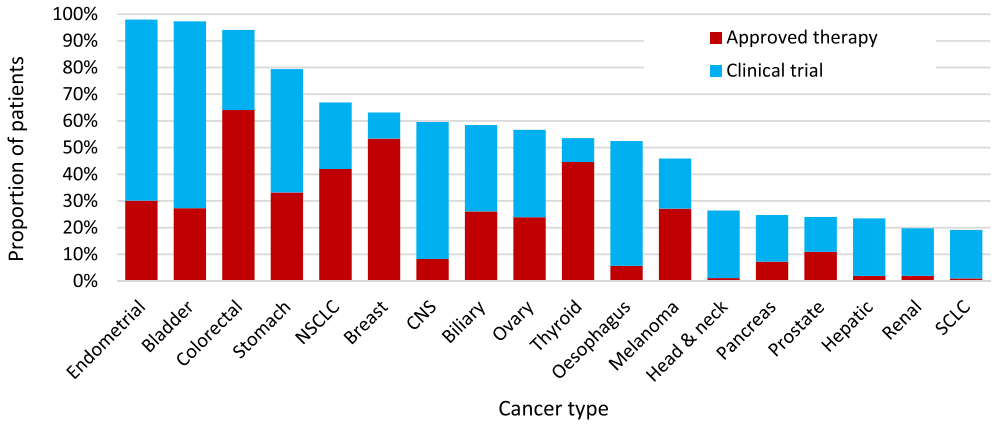


FIGURE 1 Proportion of patients with advanced cancer, by cancer type, who are potentially eligible for a biomarker-associated therapy or a biomarker-directed clinical trial. Adapted and updated from Normanno et al., 2022.[9] Biomarker prevalence data based on in-house analysis of the AACR GENIE real-world genomic data set, version 8 (AACR Project GENIE Consortium, 2017[10]). Cancer incidence based on Cancer Research UK 2015–2017. Proportion of patients with advanced disease curated from Cancer Research UK and the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) program and other sources. Approved therapy based on FDA approvals. Clinical trial biomarkers (cancer types are excluded where drugs have already been approved): ERBB2 mutation and amplification (excluding breast, NSCLC, and stomach), KRAS G12C (excluding NSCLC), CCNE1 amplification, STK11 (NSCLC only), MET amplification, PALB2 (breast, pancreas, ovary), ARID1A, EGFR (excluding NSCLC), IHD1/2 (excluding biliary), PIK3CA (excluding breast), AKT1/2/3 (excluding breast), CDK12, ERBB3/4 amplification and mutation, FGFR1 fusion and mutation, ATM (excluding prostate), BAP1, CTNNB1, NF1/2, and PTCH1. AACR indicates American Association for Cancer Research; CNS, central nervous system; FDA, US Food and Drug Administration; GENIE, Genomics Evidence Neoplasia Information Exchange; NSCLC, nonsmall cell lung cancer; SCLC, small cell lung cancer.

should be interpreted for treatment selection.[11] Historically, cancer genomics has not been part of oncology training, such that a substantial proportion of practicing clinicians struggle with understanding the clinical utility of tumor profiling and the interpretation of test results. To address this issue, dedicated precision oncology programs and molecular tumor boards have been introduced. However, these programs have yet to scale adequately to provide their expertise to the whole of the oncology community.[5] Consequently, there is a need to provide resources to practicing oncologists to improve the understanding of the value and implications of genomic profiling for cancer care.[11,12]

The objective of this review is to provide practical guidance on how to interpret and integrate genomic information into routine cancer care. We provide an overview on genomic biomarkers and their clinical relevance for therapeutic selection in patients with advanced solid tumors, describe assay technologies and sequencing processes, and offer practical advice on how to interpret and integrate molecular profiling into clinical decision making.

GENOMICS AND CANCER: BASIC PRINCIPLES

Understanding the contribution of different aberrations across the genome has advanced our knowledge of the biology of many cancer types and has led to the discovery of novel treatments for advanced cancer and personalized preventative strategies for individuals with hereditary cancer conditions. In general, cancer is driven by changes in DNA that can be genetically inherited (*germline variants*) or acquired over time (*somatic mutations*). Germline variants are inherited from parents, present at birth, usually found in the DNA of every cell in the body, and can be passed on to children. Somatic variants are not inherited from parents but rather are acquired in nongamete cells through the lifetime of an individual and cannot be passed on to children. Both somatic and germline changes in the DNA can disrupt normal cellular functions by altering the activity of individual genes (*genetic* changes) and through alterations of the cell's DNA across the genome (*genomic* changes). Although the terms *genetics* and *genomics* are often used interchangeably in oncology, they refer to different concepts: *genetics* mainly refers to understanding the structure and function a single gene or an aberration in the DNA sequence of a single gene and is usually used in the context of inherited gene variants. *Genomics* refers to the interacting structure and functional consequences of multiple genes and is mainly used in the context of somatic cancer mutations. Somatic mutations are the key drivers of malignancy and represent the most predictive biomarkers in oncology. However, around 10%–20% of patients with cancer harbor a germline variant that significantly increases their cancer risk, with some germline variants having therapeutic implications.[13,14]

Importantly, not every change in the DNA has functional consequences. On average, five to 10 genomic events are required to trigger the transformation of a normal cell into a cancerous cell.[15,16] These events are commonly referred to as *driver* mutations and can sometimes be exploited as therapeutic targets. However, the total number of mutations in any given cancer is substantial, ranging from a few thousand mutations in some breast cancers to >100,000 mutations in hypermutated colorectal and endometrial cancers.[17] Most of the genomic damage acquired by a cancer cell, therefore, is of

limited or no biological consequence, including a large proportion of mutations that fall within coding regions. These inconsequential genomic events are referred to as *passenger* mutations. Distinguishing driver from passenger mutations is a critical step in unlocking the utility of comprehensive genomic profiling.

Driver mutations occur in genes involved in essential cellular processes, such as cell growth regulation, DNA repair, cell cycle control, and cell signaling. The genes frequently mutated in cancer are broadly divided into two classes: *oncogenes* and *tumor suppressor genes*. In normal cells, proteins encoded by *oncogenes* (referred to as proto-oncogenes in normal tissue) are predominantly involved in enhancing cell proliferation and inhibiting cell death by apoptosis. During malignant transformation, genomic mutations result in increased activity. Conversely, normal proteins encoded by *tumor suppressor genes* negatively regulate cell division and ensure proper functioning of the cell. In cancer, genomic mutations result in reduced or absent activity. Classification of genes as oncogenes or tumor suppressor genes is determined by an understanding of normal cell biology and how this is disrupted in cancer. Several groups have reviewed the available evidence to generate curated lists of classified cancer genes.[18–20]

The two main classes of genomic alterations are *small* variants and *structural* variants. *Small* variants alter DNA by changing, deleting, or adding one or a small number of bases. *Structural* variants are alterations of larger stretches of DNA that result in changes in the copy number of the gene (deletion or amplification) or the transposition of large pieces of DNA from one location to another (insertions, translocations, or inversions; Figure 2).

Single nucleotide variants that alter a single DNA base in the coding region of a gene can result in a change in the amino acid sequence of the encoded protein (*missense* mutation), which may result in either loss or gain of protein function. Altered DNA bases can also lead to insertion of a premature stop codon (*nonsense* mutation), causing a truncated protein, which typically results in the loss of protein function. Small variants in which bases are added or inserted are referred to as *insertion-deletion* mutations (indels or delins). Where an indel mutation in a coding region affects a number of DNA bases that is not divisible by three, the reading frame is disrupted (*frameshift* mutation), resulting in the translation of an aberrant protein and usually resulting in loss of function. When the number of bases affected is divisible by three, the reading frame is preserved (*inframe indel*), resulting in a protein with missing or additional amino acids. Inframe indels can result in loss or gain of protein function. Base changes and inframe indels can also result in loss of the start codon or the stop codon, either of which is likely to result in loss of protein function. Single nucleotide variants and indels can also result in disruption of essential splicing sites at the exon boundaries; this usually results in loss of protein function but can occasionally give rise to an activated protein, for example, *MET* exon 14 skipping in lung cancer.[21] The different types of small variants are illustrated schematically in Figure 2; their relative frequencies are listed in Table 1.

Structural variants are large genomic rearrangements that vary in size from a few kilobases to involving most of a chromosome. There is no consensus on the size threshold at which a large indel becomes a small structural variant. The most common type of structural variant results in a change in the number of copies of a gene, resulting in gene deletion or gene amplification. These are collectively termed *copy number alterations*. Deletion of both copies of a gene (*homozygous* deletion) or deletion of one copy and disruptive mutation of the other (*mutation* + *heterozygous* deletion) results in loss of protein function. Amplification is caused by the duplication of stretched of DNA, resulting in increased copies of a gene, usually six or more. Amplifications can result in gain of activity for some oncogenes, for example, signaling receptors such as EGFR and ERBB2 (also known as HER2).

Structural variants can result in loss of protein function when part of a gene is disrupted, resulting in loss of the reading frame. This can be caused by alterations within the chromosome (*deletion*, *duplication*, or *inversion*) or alterations involving two or more chromosomes (*translocation*). Structural variants can also result in gain of protein activity through the generation of gene fusions, loss of regulatory protein domains (e.g., EGFR variant III), or increased transcription (e.g., immunoglobulin gene rearrangements in lymphoid cancers in which an oncogene hijacks the immunoglobulin promoter to drive over-expression). Different types of structural variants are illustrated schematically in Figure 2.

Beyond changes at the level of individual genes, genomic signatures have also become part of clinical sequencing reports. These are composite patterns of small variants and/or structural variants. Important examples of genomic signatures include TMB, MSI, and homologous recombination deficiency.

Figure 3 provides a pan-cancer view of clinically important information by genomic class, along with the relative contribution of composite genomic signatures. Because clinically relevant information is found in all classes of genomic events, as well as in genomic signatures, it is important that genomic assays are designed to capture all of this information.

A key concept in cancer biology, with important consequences for the clinical application of genomic profiling, is *mutation clonality*. Clonality refers to the proportion of cancer cells that carry a given mutation. *Clonal* mutations are present in all cells that make up the tumor, are present at diagnosis, and persist at progression after therapy. As such, if therapeutically relevant, clonal mutations represent attractive therapeutic targets. Subclonal mutations are only present in a subset of cancer cells. *Subclonal* mutations may be detected at diagnosis but not at subsequent relapse, or they may emerge after therapy. The concept of cancer heterogeneity refers to the presence of multiple subclonal mutations with a single cancer. It is important to note, however, that the vast majority of clinically important driver mutations in all common cancer types are clonal and, in most cancers, are stable over time as the cancer evolves, as demonstrated by independent studies.[23,24] By contrast, resistance to therapy is generally mediated by subclones (often multiple subclones in the same patient) that may be undetectable at diagnosis but

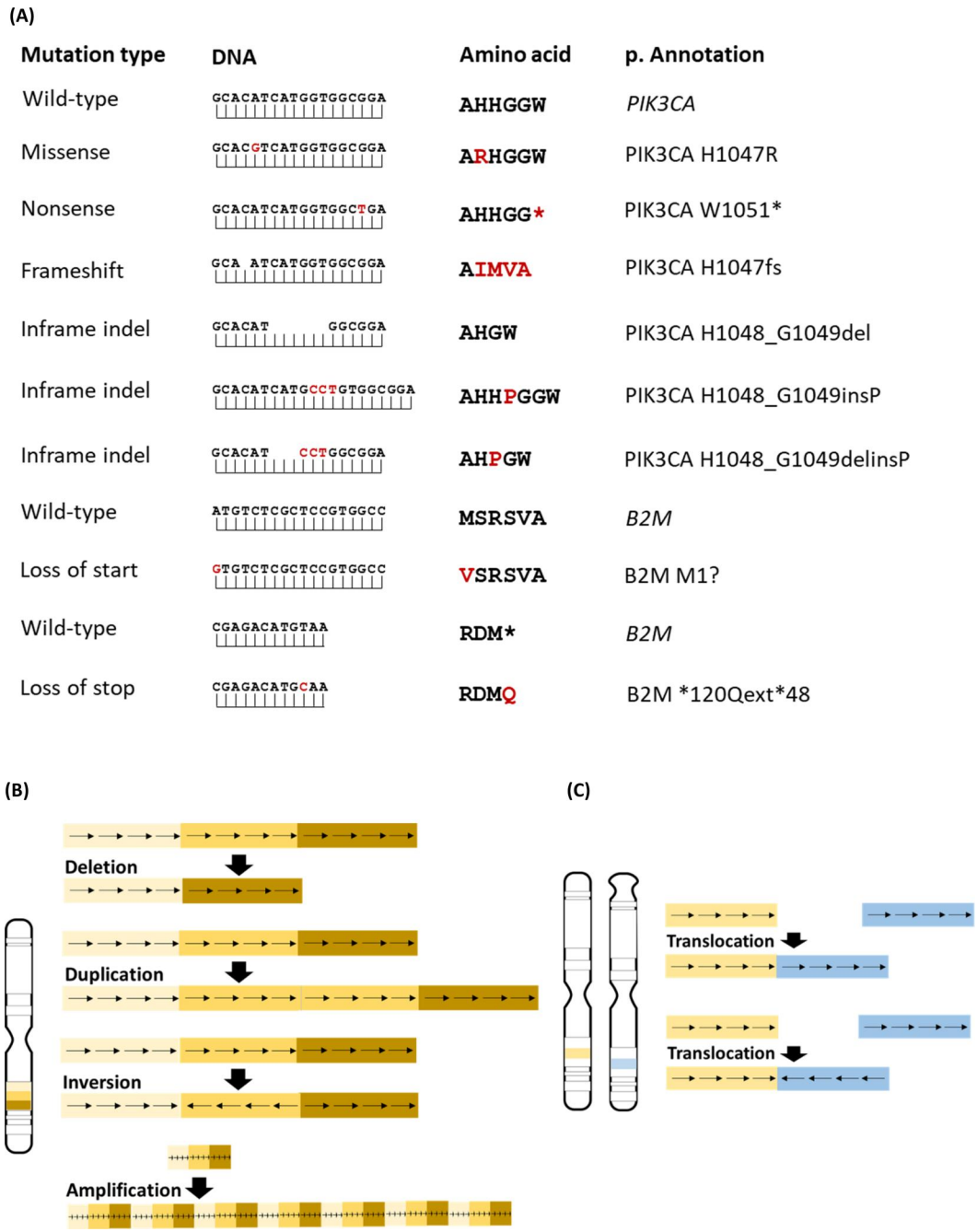


FIGURE 2 Schematic representation of different classes of genomic alteration. (A) Types and consequences of small variants. (B) Intrachromosomal structural variants. (C) Interchromosomal structural variants.

emerge at relapse after therapy.[25–29] Therefore, whereas driver mutations are nearly always clonal, emergent resistant mutations are generally subclonal.

ASSAY TECHNOLOGIES

Over the years, various technologies have been used for cancer molecular profiling, ranging from Sanger sequencing, polymerase chain reaction (PCR)-based technologies, immunohistochemistry (IHC), and fluorescence in situ hybridization (FISH), to modern NGS. Advances in assay technology have facilitated an evolution from single gene testing toward comprehensive genomic profiling. This has largely been driven by the increased availability of NGS platforms, which are able to analyze many genomic events in parallel in a manner that is tissue, time, and cost efficient. Conventional technologies are slowly being supplanted, although many retain utility in specific circumstances. Consequently, IHC and FISH still play important roles in the diagnostic toolbox, with many biomarkers currently detected by these techniques (Table 2). These are limited,

however, by their ability to detect only one alteration per assay, resulting in a lack of scalability and a need to test sequentially for different biomarkers. When several biomarkers are of potential relevance, single biomarker testing becomes both inefficient and wasteful of tumor tissue,[30] and the use of NGS becomes a more rational approach, as recommended by international cancer societies.[6,7] To guide the practicing clinician, a brief description of the principal diagnostic modalities applied in modern molecular pathology is outlined below, from traditional assays to more contemporary NGS approaches.

Conventional technologies

Direct visualization of chromosome level alterations, using FISH or cell karyotyping, is available in many clinical laboratories. FISH analysis can be used for the detection of gene fusions, deletions, and amplifications, for which it is still considered a gold-standard approach. Advantages include high accuracy and sensitivity and the ability to detect small subclonal populations of altered cells. Disadvantages include limited scalability and relatively low throughput. Karyotyping is still used to guide diagnosis and inform prognosis in some hematologic malignancies, particularly acute leukemia, although this technology is being replaced in many laboratories by targeted molecular assays, including FISH. Of interest, whole-genome sequencing (WGS) has the potential to supplant karyotyping by offering improved accuracy and efficiency.[31] NGS could potentially replace some of these assays; however, appropriate quality assurance, comparisons, and validation would need to be performed to determine accuracy and suitability.

Single gene alterations can be detected using PCR-based technologies, including real-time PCR and digital droplet PCR. These technologies have the advantage of high sensitivity and are particularly useful in the detection and monitoring of minimal residual disease. They can also offer fast turnaround times for automated systems. The main disadvantages are the limited scope of the assays (which can generally only detect mutations in one or a few genes) and their limited scope for multiplexing.[32–34]

IHC, a protein-based assay, is routinely used for the detection of biomarkers, such as expression of ERBB2 (HER2) and the estrogen receptor. IHC can also be used for the detection of specific mutant proteins (e.g., BRAF V600E) and specific gene fusions (e.g., ALK fusions) and as a screening test for some genomic alterations (e.g., NTRK fusions). IHC has the advantage of being part of routine tissue analysis pathways, such that additional tests can easily be incorporated. It can also inform on subcellular protein localization. Disadvantages include limited scalability and high tissue requirement (one tissue section per stain).[35–37]

TABLE 1 Prevalence of different types of somatic coding mutations.[a]

Mutation type	Prevalence
Missense	75.1%
Frameshift	10.4%
Nonsense	8.8%
Essential splice	3.0%
Inframe insertion/deletion	2.5%
Loss of start	0.1%
Loss of stop	0.1%

[a]Analysis of all coding mutations from Genomics Evidence Neoplasia Information Exchange (GENIE) version 10 (American Association for Cancer Research).

Next-generation sequencing technologies

NGS covers a group of deep sequencing systems able to simultaneously detect low-frequency alterations in multiple different DNA targets. NGS assays can either sequence the entire genome (WGS) or be designed to enrich for genomic areas of interest. WGS can detect all classes of genomic variants and genomic signatures across most regions of the genome. Projects like the International Cancer Genome Consortium have demonstrated the feasibility of using this technology in clinical samples, with projects ongoing to introduce WGS in clinical

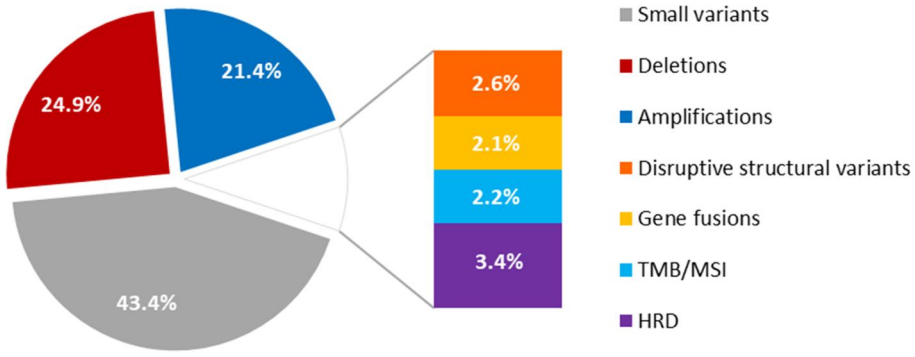


FIGURE 3 Proportion of clinically useful genomic information by mutation class. Analysis of driver mutations in 174 clinically important genes in pan-cancer analysis of whole genomes samples.[16] Adapted from Beer et al., 2020[22]. HRD indicates homologous recombination deficiency; MSI, microsatellite instability; TMB, tumor mutational burden.

TABLE 2 Gene, protein, and biomarker testing overview.[a]

Gene/protein/signature	Biomarker	Routine testing
BRCA1, BRCA2	Germline/somatic *BRCA1* and *BRCA2* mutations	NGS (DNA)
PI3KCA	*PI3KCA* mutation	NGS (DNA)
KRAS, NRAS	*KRAS/NRAS* wild type	NGS (DNA)
FGFR2/3	*FGFR3* mutations, *FGFR2/3* fusions	NGS (DNA), FISH
EGFR	*EGFR* exon 19 deletion, exon 21 L858R mutation	NGS (DNA), PCR
FLT3	*FLT3* mutations	NGS (DNA), PCR
PDGFRB	*PDGFRB* rearrangement	FISH, NGS (DNA/RNA)
MET	*MET* amplification, *MET* exon 14 alterations	FISH, NGS (DNA/RNA)
RET	*RET* fusion, *RET* mutation	FISH, NGS (DNA/RNA)
ROS1	*ROS1* translocation	FISH, NGS (DNA/RNA)
ALK	*ALK* translocation	FISH, IHC
Androgen receptor (AR)	AR expression	IHC
Estrogen/progesterone receptors (ER/PR)	ER/PR expression	IHC
BCR::ABL1	*BCR::ABL1* fusion	IHC, FISH, NGS (DNA/RNA), PCR
KIT, PDGFR	*KIT* exon 9 and 11 mutations, *PDGFR* mutations	IHC, NGS (DNA)
IDH1, IDH2	*IDH1/2* mutations	IHC, NGS (DNA)
MSI-H or dMMR	*MLH1*, *MSH2*, *MSH6*, *PMS2* protein expression, MSI high	IHC, NGS (DNA), PCR
BCL2	BCL2 protein expression, *BCL2* amplification/translocation	IHC, FISH
ERBB2	ERBB2 (HER2) protein expression, *ERBB2* amplification	IHC, FISH
NTRK	NTRK protein expression, *NTRK* fusion	IHC, FISH, NGS (DNA/RNA)
BRAF	*BRAF* V600E/K mutation	IHC, PCR, NGS (DNA)
TMB	TMB high	NGS
HRD	HRD	NGS

Abbreviations: dMMR, deficient mismatch repair; FISH, fluorescence in situ hybridization; HRD, homologous recombination deficiency; IHC, immunohistochemistry; MSI-H: microsatellite instability high; NGS, next-generation sequencing; PCR, polymerase chain reaction; TMB, tumor mutational burden.

[a]Conventional tests, such as IHC and FISH, are fundamental diagnostic tools in daily practice, with many biomarkers currently detected using these two techniques. Some conventional assays could potentially be replaced with NGS testing, such as *ERBB2* amplification; however, appropriate comparisons and validation would need to be performed.

practice (Genomics England; Personalised Oncogenomics), albeit with a requirement for labor-intensive, bespoke tissue pathways. Ongoing barriers to wider adoption include a requirement for fresh tumor material (because WGS is not yet suitable for the analysis of formalin-exposed DNA in routine clinical practice) and high costs when the whole pathway is taken into consideration (including adequate sequencing depth, need for an individual's germline control, and costs of data analysis and storage).

Targeted NGS assays comprise whole-exome sequencing and targeted gene panels. These assays use a step to enrich for regions of interest to be sequenced by using either DNA baits or PCR amplification, thus restricting the amount of sequencing required (a whole exome is 1%–2% of a genome, and even a large panel of 500 genes is only 0.1% of a genome). Although whole-exome sequencing performs well on formalin-exposed DNA, the high costs of this technology pose substantial limitations to its implementation in health care facilities.[22,38] Targeted gene panels are more affordable and represent most comprehensive genomic assays currently in clinical use. In addition to more favorable economics, targeted NGS assays have other advantages, including flexible design covering any number of genes (commonly 50–500 in clinical assays), the ability to analyze DNA and RNA, applicability to tumor and cell-free nucleic acids, automated laboratory workflows, affordable reagent costs, optimal tissue use, and the ability to capture all relevant classes of genomic alteration.[39]

There has been emerging interest in recent years in the analysis of cell-free DNA as a means of genomic profiling for those living with cancer. Both cancer cells and normal cells shed DNA into the bloodstream. DNA shed from cancer cells, referred to as *circulating tumor DNA* (ctDNA), can be analyzed to detect the mutations present in the tumor cells. The ability of NGS assays to analyze ctDNA from serial blood tests, often referred to as liquid biopsies, allows for monitoring of tumor dynamics and treatment response over time.

Studies are currently underway to assess the clinical utility of ctDNA.[40,41]

Assay selection: NGS versus routine pathology testing

The choice of molecular profiling technology depends on the specific clinical questions to be addressed because each method has its strengths and weaknesses. Most genomic biomarkers associated with approved therapies can be determined by single-gene/few gene tests performed in the routine pathology laboratory using FISH (gene fusions), IHC (some gene fusions, some missense mutations), or single-gene/few gene PCR-based assays (Tables 2 and 3). This approach is reasonable when small numbers of biomarkers are tested in a few patients, but it does not scale efficiency as throughput increases. Demand for more biomarkers per patient and greater patient throughput eventually results in increased costs, longer turnaround times, and exhaustion of available tissue for testing. Tissue exhaustion is a particular issue in some tumor types, such as lung cancer, in which tissue samples are difficult to acquire and there are multiple biomarkers that need to be tested. Ultimately, there is a tipping point in different cancer types at which the number of biomarkers per patient and the number of patients to be tested favors NGS over routine pathology testing, with NGS delivering more efficient use of tissue, lower costs, and faster turnaround times. This tipping point has already been reached for lung cancer, in which NGS has clear benefits over routine pathology testing, is close to this point for colorectal cancer, and will be reached soon for several other cancer types as more biomarker-directed therapies are approved.[42–44]

Overall, there is evidence of comparable sensitivity and specificity in identifying prevalent, treatable genomic abnormalities in routine clinical use between conventional technologies and NGS. The falling costs and improvements in turnaround times for NGS, along with advances in bioinformatics analyses and the standardization of knowledge bases for clinical interpretation of genomic findings, collectively present a persuasive case for adopting NGS in precision cancer care. Because of the approval of five tumor agnostic biomarkers by the US Food and Drug Administration (comprising NTRK fusion, MSI, high TMB, *BRAF* V600E mutation, and RET fusion), multigene sequencing should be preferred for patients with advanced

TABLE 3 Conventional technologies for molecular profiling.

Technique	Description	Pros	Cons	Current utility
Sanger sequencing	Traditional DNA sequencing method that involves chain termination during DNA synthesis using dideoxynucleotides, allowing the determination of the DNA sequence	It is considered reliable for short DNA sequences, providing accurate, base-by-base sequencing results	Targeted approach Time-consuming and relatively expensive, limiting its utility for large-scale genomic analysis	Suitable for validating specific mutations or analyzing specific genes
PCR	Used to amplify a specific DNA segment; it is often combined with Sanger sequencing for targeted analysis of genes or mutations	Highly sensitive and can amplify small amounts of DNA for further analysis	Is a targeted approach, which means it can only analyze specific regions of the genome, limiting its scope	Useful when studying known mutations in specific genes or when a limited number of genes need to be analyzed
IHC	Uses antibodies to detect specific proteins in tissue sections, helping identify the presence or absence of certain biomarkers	Allows the visualization of protein expression and localization in the context of tissue morphology	Provides qualitative rather than quantitative data, and the quality of results can be affected by the quality of antibodies and tissue processing	Routinely used to identify the tumor origins and for diagnostic biomarker assessment Is valuable for assessing protein expression in clinical tumor samples, thus determining the presence of specific therapeutic targets
FISH	Uses fluorescent probes to bind to specific DNA sequences, allowing visualization and detection of chromosomal abnormalities or gene amplifications	Powerful tool for detecting specific genetic alterations and assessing gene copy number changes in individual cells Traditionally used as confirmatory strategy in PCR-positive or IHC-positive cases (ALK, ROS1, RET, NTRK) or to validate challenging NGS results	Its utility is limited to predefined targets and does not provide comprehensive genomic information	Useful for the identification of specific gene rearrangements, gene amplifications, or deletions Commonly used in the molecular characterization of certain cancers like breast cancer, hematologic malignancies, and sarcoma

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; PCR, polymerase chain reaction.

solid tumors even if only a single approved genomic biomarker-linked treatment is available. Multigene panel-based testing may also identify genomic alterations that suggest the patient may benefit from a clinical trial. This is particularly important for younger patients, for whom access to novel therapies may represent their best chance of long-term survival.

In summary, NGS is the most powerful tool for comprehensive genomic analysis and is tissue, time, and cost efficient. The decision to use NGS over IHC/FISH (or in addition to these methods) depends on several factors (Table 4). It is essential to consider the specific clinical context, the goal of the molecular profiling, and the available resources when deciding whether to use NGS or other assays. In some cases, IHC/FISH may be sufficient to identify specific actionable mutations, especially when the target is well established and validated (e.g., estrogen receptor, progesterone receptor, and ERBB2 in breast cancer). NGS is valuable for clinical applications that require a more comprehensive and exploratory approach to identify a broader range of genomic alterations and potential therapeutic targets, and it is tissue and cost efficient. In addition, NGS can be considered for cases in which IHC/FISH results are equivocal or inconclusive, providing an additional layer of information for more confident decision making.

GENOMIC PROFILING: OVERVIEW OF TESTING INDICATIONS

The identification of biomarkers and the development of new targeted therapies are evolving rapidly over time as new evidence emerges. For clinicians, it is important to stay up to date on the latest

TABLE 4 Next-generation sequencing versus immunohistochemistry and fluorescence in situ hybridization.

Scenarios in which NGS might be preferred	Considerations
Comprehensive genomic profiling	NGS provides a comprehensive view of the cancer genome, allowing the simultaneous analysis of multiple genes and genomic alterations. It can identify not only known mutations but also novel and rare mutations that may not be covered by IHC/FISH panels. If there is a need to analyze a large number of genes or the entire exome/genome for comprehensive profiling, NGS is the preferred choice.
Identification of novel biomarkers	IHC and FISH are limited to predefined targets and may not cover emerging or recently discovered biomarkers. NGS can help identify new actionable mutations and potential therapeutic targets beyond the known ones, offering a more exploratory approach to biomarker discovery.
Assessment of tumor heterogeneity	NGS provides insights into tumor heterogeneity, in which different regions of the same tumor may have distinct genetic alterations. IHC and FISH often represent an average result for the entire tumor section, whereas NGS can identify spatial and temporal genomic variations within the tumor.
Detection of fusion genes and structural variants	NGS is more suitable for detecting fusion genes and complex structural variations, which are not easily identified using IHC/FISH methods.
Tumor mutational burden (TMB) and microsatellite instability (MSI)	NGS allows the estimation of TMB and MSI, which are emerging biomarkers for immunotherapy response prediction. These markers are not assessable through IHC/FISH.
Liquid biopsies and circulating tumor DNA (ctDNA) analysis	NGS can be used to analyze ctDNA in liquid biopsies, allowing monitoring of tumor dynamics and treatment response over time. IHC/FISH are not applicable for this purpose.
Molecular profiling across cancer types	NGS is a versatile platform that can be used for molecular profiling across various cancer types, making it more cost effective and efficient compared with running multiple IHC/FISH tests for different markers.
Scenarios in which FISH might be preferred	**Considerations**
Very low tumor cellularity or identification of subclones important	FISH is able to detect alterations in which only a small number of cells is present, either because of low tumor cellularity or small subclonal populations.
Scenarios in which IHC might be preferred	**Considerations**
Lack of NGS infrastructure	IHC is widely available in clinical laboratories.
New biomarker	Where IHC is applicable, novel biomarkers can be quickly introduced into routine analysis pathways, whereas inclusion in an NGS assay may take longer because of assay redesign and validation.

Abbreviations: FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing.

expert guidelines and recommendations from cancer societies in the field. International guidelines differ slightly in their recommendations concerning which patients should be offered genomic profiling, and comprehensive details on genomic testing indications for therapeutic selection in oncology lie beyond the scope of this article.[6,7] Overall, there is general agreement on performing molecular profiling at the time of presentation with metastatic or locally advanced cancer when approved biomarker-associated therapies are available. Significant proportions of patients with many common cancer types are now eligible for a biomarker-associated, targeted therapy (Figure 1). A few biomarker-associated therapies have recently gained approval in the adjuvant setting, further expanding the indications for genomic profiling to patients with early stage disease in selected tumor types.[24,45] Clinical judgement is an essential component of patient selection for testing, and genomic profiling should not be ordered when the test result will most likely not influence the clinical management.

In addition to tumor genomic profiling for somatic mutations, germline genetic testing is recommended for several cancer types and has been shown to have a positive impact on survival.[46,47] The detection of a germline variant may have direct consequences on the treatment of the cancer, for example, *BRCA1* and *BRCA2* mutations in the management of breast, ovarian, prostate, and pancreatic cancer.[48,49] The detection of a germline variant may also have consequences for the screening and prevention of future cancers in the patient and for genetic testing of family members to determine whether they carry the same variant through so-called *cascade testing*. Patients potentially eligible for germline testing are usually identified according to standardized criteria relating to factors such as family history of cancer, age of diagnosis, and cancer type. However, many patients with germline pathogenic variants (up to 50%) do not meet the conventional criteria for germline testing based on family history.[13,14,50,51] Therefore, to maximize the identification of mutation carriers, the US National Comprehensive Cancer Network has updated their guideline recommendations suggesting that all patients, or a high proportion of patients, with a personal history of breast, ovarian, endometrial, pancreatic, colorectal, or prostate cancer should undergo germline genetic testing.[46,47] In addition to preventative implications, this is particularly important given the availability of approved therapies targeting germline genetic alterations in some of these tumor types.

With regard to the timing of genomic profiling, because the vast majority of clinically meaningful driver mutations are clonal and, in most cancers, are relatively stable over time as the cancer evolves,[23,24] it is reasonable to perform tumor genomic profiling at the time of diagnosis with advanced disease. For subsequent genomic profiling, particularly after molecularly targeted therapy, analysis of ctDNA has a potential advantage over tumor tissue DNA because plasma DNA can comprise tumor DNA from all sites of disease. This is important given the subclonal nature of emergent-resistance mutations, particularly when these resistance mutations can be used to guide subsequent lines of therapy, for example, in lung and breast cancers.[52–55]

Serial analysis of ctDNA after therapy may be able to predict treatment response in some tumors and identify disease progression earlier than standard imaging approaches[56, 57]; however, protocols to use these data to guide therapy decisions are yet to be established, and the use of ctDNA analysis in this setting is not recommended outside the context of a clinical trial. Analysis of ctDNA is also showing emerging promise in the detection of minimal residual disease after potentially curative surgery for localized malignancies.[58–62] In this setting, detection of ctDNA after surgery is a strong predictor of subsequent disease progression and may be useful in deciding whether adjuvant chemotherapy is likely to be beneficial in cancers like breast and colorectal. Currently, the use of ctDNA to guide adjuvant therapy treatment decisions is not recommended outside the context of a clinical trial. The use of ctDNA to guide therapeutic selection is likely to increase over the coming years as more molecularly targeted therapies reach the clinic and our understanding of genomically mediated resistance evolves and expands. A general overview of genomic test selection for patients with advanced cancer is illustrated in Figure 4.

CANCER GENOMIC PROFILING: FROM DNA EXTRACTION TO VARIANT INTERPRETATION

To understand and interpret a cancer genomic profiling report, it is important to have a basic knowledge of how the sequencing process works, including how data that are generated are processed and analyzed. The sections below provide a physician-focused overview of the key aspects of cancer genomic profiling, from DNA sequencing to assessment of actionability of genomic findings.

Data process and driver annotation

The first step of the genomic sequencing process is *DNA extraction* from the patient sample, library preparation, and generation of raw sequencing data. The raw data require extensive processing, which includes filtering of poor-quality data, alignment to a reference genome, determination of different classes of genomic alteration (small variants, structural variants, etc.) by different analysis algorithms, determination of impact on protein translation, and filtering of germline variants.

The next step is *driver annotation*, which aims at distinguishing the genomic events that drive neoplastic change (driver events) from inconsequential genomic damage (passenger events). In broad terms, driver mutations result in loss of tumor suppressor gene activity or gain of oncogene activity. Small variants that result in loss of tumor suppressor gene activity include nonsense, frameshift, and essential splice mutations and, less frequently, loss of start, loss of stop, or large inframe indel mutations. In addition, missense mutations and small inframe indels can result in loss of tumor suppressor gene activity when they affect important functional domains. Structural variants resulting in loss of tumor suppressor gene activity include partial or

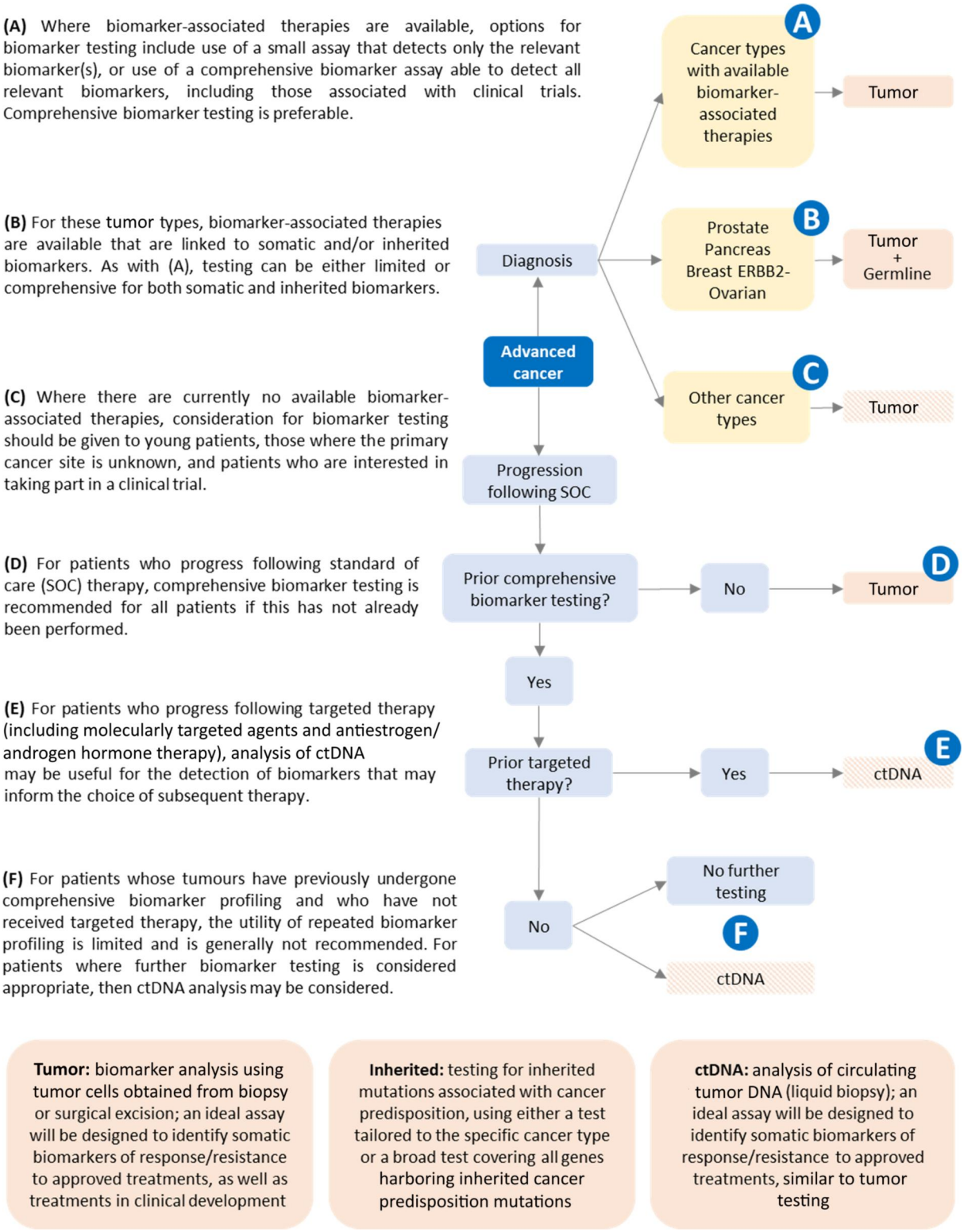


FIGURE 4 Overview of genomic test selection for patients with advanced cancer, at diagnosis and at progression following standard of care, for informed preventative and therapeutic decision-making. ctDNA indicates circulating tumor DNA; SOC, standard of care.

complete gene deletion and other genomic alterations resulting in disruption of the gene's reading frame (Figure 2). Small variants that result in gain of oncogene function comprise missense mutations and occasionally inframe indels, which target important protein domains. In rare instances, protein truncation or splice-site mutations can result in activation of an oncogene. Structural variants resulting in gain of oncogene activity include gene fusions, partial gene deletions (e.g., EGFR variant III), and partial gene duplications (e.g., FLT3-ITD).

Clinical laboratories differ in how they report driver and passenger mutations. Some laboratories include only driver mutations in the report, whereas others include a list of passenger mutations in a separate section or as an appendix to the report. Assigning driver versus passenger status to individual mutations is not always a straightforward process. Whereas nonsense and frameshift mutations resulting in disruption of a tumor suppressor gene can generally be assigned driver status with a high degree of confidence, missense mutations in either tumor suppressor genes or oncogenes can be more difficult to classify. Because missense mutations account for 75% of all coding mutations in cancer (Table 1), annotation of this variant class can present a significant challenge to the efficient reporting of comprehensive cancer assays. Several sets of guidelines, created by national and international groups,[19,63–66] have been developed to address the complexities of driver annotation. These guidelines are broadly aligned in following a data-gathering approach to driver annotation, which involves the amalgamation and assessment of data from different sources to reach a conclusion about driver status for each individual mutation. The recommended data sources used include databases of clinical samples (to assess how many times the mutation has been reported), curated databases of cancer mutations (Table 5), in silico protein prediction tools (to assess the likely impact of the mutation on protein function), and parsing of published literature for information on the biological impact of the mutation.[19,63–66] Additional resources include mathematical modelling of clinical samples to predict driver mutation hotspots.[67–70] A current challenge in clinical practice is how to integrate the outputs of these various data sources to deliver an objective, accurate, and reproducible methodology for determining driver status for each individual mutation. To date, there is no gold-standard methodology for driver annotation, with clinical laboratories using variations of the assorted published guidelines.

Clinical interpretation of genomic variants

The next step after differentiating driver mutations from passenger events is to assess the *clinical significance*, i.e., the potential diagnostic, prognostic, or therapeutic implications, of the identified variants. Multiple resources, including publications from professional societies and curated knowledge bases, can potentially address the actionability of somatic cancer variants and provide rules for their curation. The Precision Oncology Knowledge Base (OncoKB), developed by the Memorial Sloan Kettering Cancer Center, classifies the possible therapeutic applications of the variants into four levels, to which is added a fifth subgroup of variants associated with resistance to therapies.[20] Joint recommendations of the Association for Molecular Pathology, ASCO, and CAP categorize somatic variants based on four tiers.[65] Tier I includes variants with strong clinical significance, and tier II includes those with potential clinical significance, whereas tier III contains variants of unknown clinical significance, and tier IV contains benign or likely benign variants. Only tier I and II variants have implications for therapeutic intervention. Finally, the ESMO Scale of Clinical Actionability for Molecular Targets defines six levels of clinical evidence for molecular targets.[71] Tier I includes the targets ready for implementation in routine clinical practice, and tier II includes the investigational targets that are likely to predict benefit from a targeted drug, although additional data are needed. Tiers III–V comprise targets with lower levels of evidence, and tier X comprises targets that lack of evidence for actionability.

TABLE 5 Academic and commercial precision oncology knowledge bases.

Knowledge base	Revenue model	Free to access?	Affiliated institute
OncoKB	Hybrid	Yes (if registered)	Memorial Sloan Kettering Cancer Center, US
Knowledge Base for Precision Oncology	Hybrid	Yes (partial if registered)	The University of Texas MD Anderson Cancer Center, US
Clinical Interpretation of Variants in Cancer (CIViC)	Academic	Yes	Washington University St Louis, US
Database of Curated Mutations (DoCM)	Academic	Yes	Washington University St Louis, US
MyCancerGenome	Hybrid	Yes (partial if registered)	Vanderbilt University, US
Cancer Genome Interpreter	Academic	Yes	Barcelona Biomedical Genomics Laboratory, Spain
Clinical Knowledgebase (JAX-CKB)	Commercial	Yes (partial if registered)	Jackson Laboratories, US
Precision Medicine Knowledge Base (PMKB)	Academic	Yes	Weill Cornell Medicine, US
PierianDx	Commercial	No	NA
Qiagen	Commercial	No	NA
Foundation Medicine	Commercial	No	NA

Abbreviation: NA, not applicable.

READING AND INTERPRETING AN NGS REPORT

The complexity of biomarker testing is increasing, resulting in clinical reports, sometimes from multiple sources, that contain a large amount of information that can be difficult to read and interpret.[72] Before assessing the genomic variants identified through genomic profiling, an initial overall assessment of the data included in the report is an important first step to ensure that the information is suitable for clinical use. There are some important aspects to consider to critically analyze the general quality of a cancer genomic report.

The sample

Tumor tissue is the preferred material for initial genomic profiling. This can be derived from biopsy, surgical resection, or cytology sampling. Pathologic assessment is helpful to determine the tumor cellularity.[73] On average, biopsy samples from solid tumors contain around 30% tumor cells. For some tumor types, for example, pancreatic cancer, tumor cellularity is often much lower, which will impact the utility of genomic profiling by diluting out the signal from the malignant cells. Where no driver mutations have been detected, consideration should be given to low tumor cellularity, in conjunction with a review of the pathology sections (bearing in mind that tumor cellularity estimates based on morphology are not very reliable). Where small variant driver mutations have been detected but variant allele fractions (VAFs) are close to the limit of detection for the assay, there is the possibility that additional driver events may not have been detected and that genomic signatures like TMB may be underestimated. Samples in which the tumor cellularity is low may be amenable to tumor enrichment, for example, by macrodissection of tumor-containing areas. It is important to note that an assessment of copy numbers from NGS samples requires the tumor cellularity to be known.

Next-generation sequencing assay

It is important to check whether the biomarkers that need to be assessed in the patient according to international and/or national guidelines are covered by the assay because NGS panels are highly variable in terms of the number of genes or genomic regions covered and the type of genomic alterations analyzed (mutations, copy number alterations, and rearrangements/fusions). It is also important to verify whether the panel is based on DNA or RNA sequencing for detecting fusion genes because of the different sensitivity associated with the different sources of nucleic acids.[74] The description of the sequencing method in the report should also state whether a commercial assay or a laboratory-developed test was used, including whether validation of the method has been performed. Laboratories should record International Organization for Standardization certifications and participation in external quality-assurance programs because this information helps to reassure the clinician about the quality of the test performed.[75]

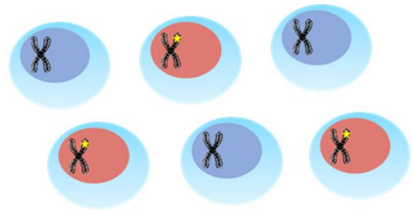
Variant allele fraction

The variant allele fraction should always be disclosed. VAF is an important (and often misunderstood) characteristic of a somatic mutation and represents the number of DNA molecules in the sample that harbor the mutation as a proportion of all DNA molecules, mutant and wild-type. VAF reflects a composite of three factors: tumor cellularity, mutation clonality, and allelic imbalance. Figure 5 illustrates some worked examples of the impact of tumor cellularity, mutation clonality, and allelic imbalance on VAF. Most NGS panels have a VAF-based limit for reporting identified variants, which differs between tissue and ctDNA tests (typical cutoffs for tumor and ctDNA are 5% and 0.1%, respectively). The VAF of each variant should be compared with the neoplastic cell fraction (for tumor samples) and to the VAF of other variants in the sample to assess whether the mutation is clonal or subclonal. In the case of ctDNA analysis, the VAFs of the identified variants are very often low, and it is more difficult to establish their clonal or subclonal origin because the real fraction of tumor DNA is not known, and different tumor clones may release ctDNA to a different extent.[77] Reassuringly, most studies have described very similar response rates in patients who were analyzed using tissue-based or ctDNA-based assays and were treated with targeted therapies. It is important to remember that accurate VAFs cannot be calculated for structural variants such as gene fusions (using DNA or RNA).

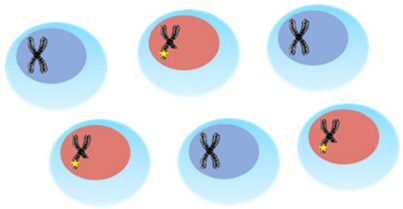
Germline variants and the importance of reflex germline genetic testing

In most clinical settings, up-front genomic profiling of cancer samples is performed on tumor tissue only. Therefore, careful consideration is required for the management of potential germline variants detected by tumor-only sequencing in patients who are yet to undergo germline testing. Potential germline variants warrant confirmation by testing of a constitutional sample, such as blood or saliva. However, overuse of confirmatory germline testing can cause considerable diversion of clinical and laboratory resources with questionable clinical benefit[78] as well as generating stress and anxiety for patients. Thus the selection of variants for confirmatory germline testing requires a balanced and data-driven approach, as elaborated in recent guidance from the ESMO Precision Medicine Working Group,[79] as summarized below.

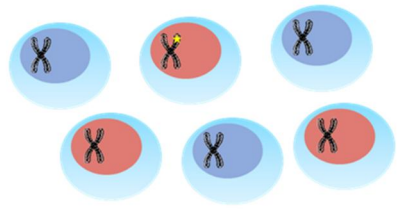
When a potential pathogenic germline variant is detected in the tumor sample, formal manual confirmation should be performed by a diagnostic scientist using an established classification framework.[80] This involves classifying each potentially germline mutation into one of five standard categories, comprising *pathogenic*, likely pathogenic, *uncertain significance*, *likely benign*, or *benign*. Confirmatory germline



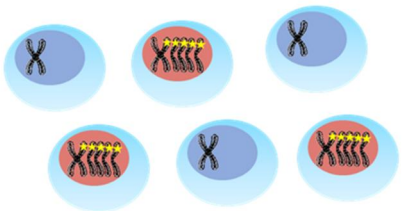
Variant allele fraction	25%
Tumor cellularity	50%
Mutation clonality	Clonal
Wild-type allele retained	



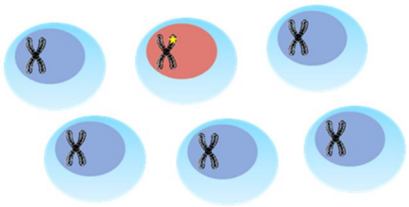
Variant allele fraction	33%
Tumor cellularity	50%
Mutation clonality	Clonal
Wild-type allele lost	



Variant allele fraction	8%
Tumor cellularity	50%
Mutation clonality	subclonal
Wild-type allele retained	



Variant allele fraction	63%
Tumor cellularity	50%
Mutation clonality	Clonal
Mutant allele amplified, 5 copies	



Variant allele fraction	8%
Tumor cellularity	17%
Mutation clonality	Clonal
Wild-type allele retained	

FIGURE 5 Worked examples of how of tumor cellularity, mutation clonality, and allelic imbalance affect variant allele fraction (VAF). Tumor biopsies contain a mixture of cancerous and noncancerous cells, with the latter comprising stromal tissue, blood vessels, and infiltrating immune cells. In samples with high tumor cellularity, the majority of sequencing reads will originate from tumor cells, making it easier to detect mutations with higher VAF. However, in samples with lower tumor cellularity, sequencing reads from normal cells can dilute the signal of tumor-related variants, leading to lower VAF. Detecting low-frequency variants becomes challenging in samples with low tumor cellularity. Within the cancer, some mutations are present in all malignant cells (clonal mutations), whereas some are present in only a subset of cells (subclonal mutations). Clonal mutations tend to have higher VAF, whereas subclonal mutations have lower VAF. Allelic imbalance refers to an unequal distribution of alleles (variants of a gene) between tumor and normal cells. This can arise because of loss of the wild-type allele or amplification of the mutant allele. Allelic imbalance is common with mutations affecting tumor suppressor genes in which the wild-type allele is often deleted, resulting in a hemizygous mutation with a consequent increase in the observed VAF. Allelic imbalance can affect VAF by skewing the proportion of variant alleles relative to reference alleles. In regions with allelic imbalance, the VAF may be higher or lower, depending on the direction of the imbalance (Yu et al., 2017[76]).

testing should be undertaken for mutations formally classified as pathogenic or likely pathogenic (but not for other variants, including those of uncertain significance). Only pathogenic and likely pathogenic variants have clinical implications in terms of cancer susceptibility and prevention and therapeutic selection.

For seven high-penetrance and highly actionable genes, when pathogenic mutations are detected by tumor-only sequencing, confirmatory germline testing is recommended in all cases, regardless of the tumor type. For an additional 33 genes, confirmatory germline testing is recommended only when the gene is associated with an increased risk of the cancer type in question; in some cases, the age of the patient at cancer diagnosis is also considered (Table 6). For all of the genes listed in Table 6, at least 5% of all qualifying pathogenic variants detected by tumor-only sequencing will be germline in origin; for genes like as *BRCA1*, *BRCA2*, and *PALB2*, this proportion is >80%.

Germline variants detected in tumor samples would be anticipated to have a VAF of approximately 50% (for heterozygous events). The VAF may be higher in the tumor when the wild-type allele has been deleted in the cancer cells. Variants with a low allele fraction (<30% for substitutions, <20% for indels) are unlikely to be germline in origin and do not warrant germline testing, as demonstrated in a recent analysis of cancer samples for which paired tumor and normal sequencing was available.[79]

Sequencing data from ctDNA

Liquid biopsy is increasingly being used to determine the tumor genomic profile for patients with advanced cancer. Overall, the analysis of data from ctDNA follows the same basic principles as data from tumor biopsy samples with a few important differences, as outlined below, but is not considered standard of care across the full spectrum of cancer care.

The key limitation of ctDNA is reduced sensitivity for driver mutations compared with analysis of tumor material. Comparative studies have consistently shown that ctDNA does not detect 20%–30% of mutations present in the tumor[81–88] and may underestimate TMB.[85] These false-negative results reflect low levels of ctDNA in some patients (with limited-stage disease and low tumor burden), and some specific tumor types are associated with less shedding of tumor DNA into the circulation.[40,41,81] It may be possible to define a threshold, based on the absolute level of tumor DNA detected in the plasma, above which false-negative results are unlikely to arise.[89,90] In patients with lower levels of ctDNA, a negative result cannot reliably exclude the presence of a mutation, such that repeat biopsy should be considered where analysis does not identify driver mutations.

Two specific factors should be noted in the interpretation of sequencing data from ctDNA: VAF and *clonal hematopoiesis*. In addition to being affected by allelic imbalance and mutational clonality, VAF is significantly affected by dilution of the ctDNA with normal DNA released from other cells in the organism. When only a small amount of tumor DNA is shed into the circulation, VAFs will be low, often <1% (depending on the sensitivity of the assay), but may nonetheless represent clonal mutations in the tumor. Thus biomarkers of response to therapy, such as a relevant *EGFR* mutation in lung cancer, should be considered clinically actionable even if the VAF is low. Clonal hematopoiesis, also referred to as *clonal hematopoiesis of indeterminate potential*, represents an expansion of hematopoietic cells harboring leukemia-associated driver mutations in otherwise healthy individuals. Clonal hematopoiesis is common in

TABLE 6 Recommended genes for confirmatory germline testing of pathogenic variants detected by tumor-only sequencing.[a]

Confirmatory germline testing recommended	Genes		
Pathogenic variant detected in any tumor type	*BRCA1*	*MLH1*	*RET*
	BRCA2	*MSH2*	
	PALB2	*MSH6*	
Pathogenic variant detected in a tumor type with an established tumor-gene association	*BRIP1*	*TMEM127*	*FLCN*
	MUTYH	*TSC2*	*NF1*
	PMS2	*VHL*	*PTCH1*
	RAD51C	*ATM*	*POLD1*
	RAD51D	*BAP1*	*POLE*
	SDHAF2	*BARD1*	*SDHA*
	SDHB	*CHEK2*	*SMAD3*
	SDHC	*DICER1*	*SMARCB1*
	SDHD	*FH*	*SUFU*
Pathogenic variant detected in relevant tumor type	*APC*	*RB1*	*CDKN2A*
Patient diagnosed with cancer at age <30 years	*PTEN*	*TP53*	*SMARCA4*

[a]Based on guidelines from the European Society for Medical Oncology Precision Medicine Working Group (Kuzbari et al., 2023[79]).

older adults, and its prevalence appears to be increased in patients with cancer, particularly those who have received prior chemotherapy,[91] such that clonal hematopoiesis mutations are frequently detected in analyses of plasma DNA.[92] The most frequent clonal hematopoiesis driver mutations arise in genes like *TET2* and *DNMT3A*, which are rarely mutated in solid tumors, such that distinguishing clonal hematopoiesis from a ctDNA cancer mutation is straightforward. However, clonal hematopoiesis may also be driven by mutations in genes that are also mutated in solid cancers, such as *TP53* and *ATM*, which can render interpretation of plasma DNA results difficult. As an example, in a study of patients with prostate cancer, potentially clinically actionable mutations detected by plasma DNA analysis were derived from clonal hematopoiesis in 10% of cases.[93] When the tissue source of a mutation is in doubt, options include sequencing of tumor-derived DNA (where available) or sequencing of DNA-extracted circulating blood cells (to rule out clonal hematopoiesis).

Integration of genomic findings into clinical care

The last step of the process is the integration of genomic findings into the patient's treatment plan to make informed decisions about the most appropriate and personalized treatment options for the patient (Figure 6). The sequencing results need to be carefully interpreted in the context of the patient's overall clinical, personal, and familial history. In addition, not all genetic alterations identified may have clinically actionable implications, and treatment decisions should be made based on a combination of genomic information, clinical expertise, and evidence-based guidelines.

Potential clinical utility of genomic biomarkers falls into one of the following four categories:

1. Biomarker-associated therapy approved for use in the tumor type. Testing may identify a predictive biomarker associated with a specific therapy. Biomarker-associated therapies may be cancer type-specific (e.g., an EGFR inhibitor for lung cancer harboring an appropriate *EGFR* mutation) or tumor type-agnostic, in which the biomarker predicts response to a drug regardless of the tumor type. Current tumor-agnostic biomarker–drug indications include NTRK inhibitors for NTRK-fusion positive cancers, combined BRAF and MEK inhibition for tumors harboring a *BRAF* V600E mutation (excluding colorectal cancer), and immune checkpoint inhibition for tumors with MSI or high TMB. It is important to note that drug approvals and reimbursement vary by geography; although timelines for regulatory approval are similar in the United States and the European Union,[94] Europe often lags behind the United States when it comes to drug availability,[5] likely reflecting differences in the timing of regulatory company filings and pathways to reimbursement in the two geographies. International initiatives are looking to align national regulators to develop faster and more efficient pathways to drug approval.[95,96]
2. Biomarker-associated therapy approved in another tumor type. There are biomarker-associated therapies approved in specific

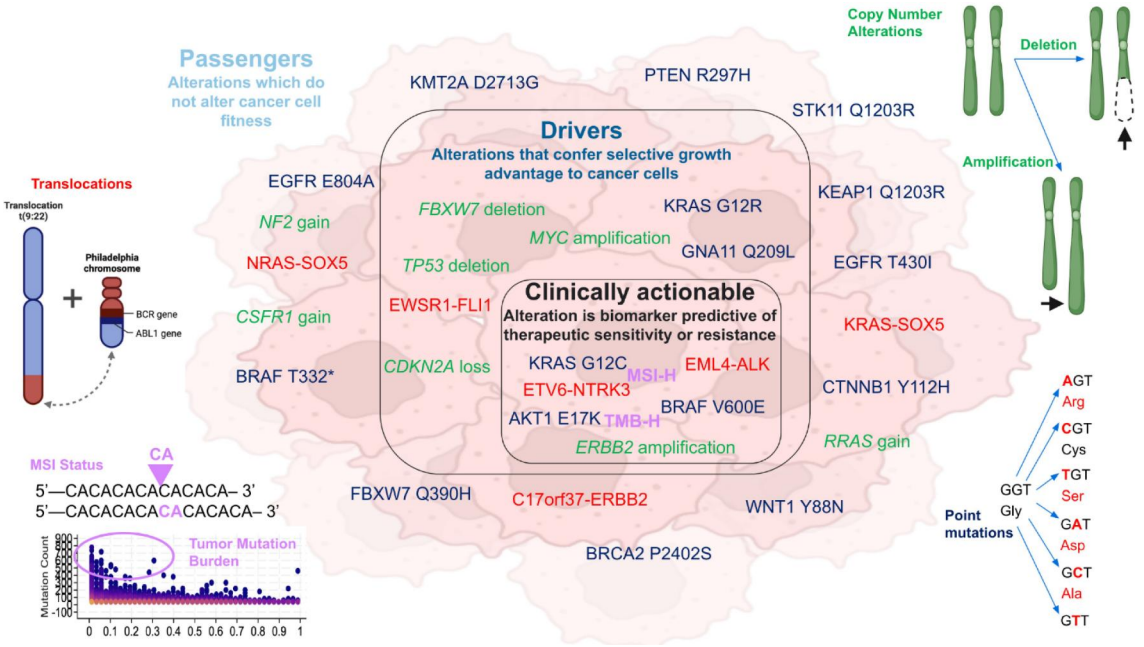


FIGURE 6 Overview of the analysis of genomic cancer data. Analyzing genomic cancer data is a critical aspect of understanding the molecular underpinnings of cancer and developing targeted therapies. This process involves identifying genetic alterations that drive cancer progression (driver mutations) as well as alterations that are incidental to cancer development (passenger mutations). Copy number alterations, comprising deletions and amplifications, are also crucial genomic events that play a significant role in cancer biology, as are chromosomal translocations. The analysis of genomic cancer data requires a combination of advanced sequencing technologies, computational tools, and experimental validation. MSI indicates microsatellite instability; orf, open reading frame.

tumor types that have efficacy in additional indications; for example, ALK fusions in tumors other than lung cancer.[97,98] These biomarkers may be included in expert guidelines, such those from the National Comprehensive Cancer Network, ASCO, or ESMO. Frequently, these biomarkers are found in rare cancer types or in very small subpopulations of more common cancers, which has precluded the conducting of an appropriate clinical trial. When a clinical trial is available, offering patients enrolment is the preferred option, such that efficacy and safety data can be collated. Where there is no clinical trial option, the use of off-label therapy may be appropriate, particularly in young and fit patients.

3. Potential for a biomarker-directed clinical trial. There is a growing appreciation that early phase clinical trials represent a valid therapeutic option for individuals living with cancer who have exhausted standard-of-care therapies.[99–101] Importantly, biomarker-based treatments are more likely to yield clinical responses, with the use of a genomic biomarker delivering mean response rates of 40% in a meta-analysis of phase 1 oncology trials.[102] Comprehensive biomarker profiling represents an efficient approach to the identification of potential clinical trial biomarkers, with local expertise within the molecular tumor board, combined with the use of relevant search tools (Table 7), enabling the identification of a suitable clinical trial.
4. Preventative and therapeutic implications of germline variants. Germline pathogenic and likely pathogenic variants may have clinical implications in terms of both therapeutic selection and cancer susceptibility and prevention. In the event of the identification of a variant in a gene potentially associated with hereditary cancer syndromes through tumor-only sequencing, the patient should be referred to genetic counseling (or other trained health care providers) to discuss the need to perform a germline genetic test to verify the somatic or germinal nature of the identified variant.[103] When a germline variant is confirmed, genetic counseling and germline testing should be extended to family members to identify those carrying the same genetic alteration, who can be referred for risk-reducing surgery or early detection strategies (to reduce the risk of cancer and detect the cancer earlier, respectively).

It is important to note that clinical actionability changes over time with the introduction of new drug approvals and biomarker-directed clinical trials. Because of these considerations, the identification of biomarkers as clinically actionable requires the integration of regulatory approval (which may be geography-specific), professional guidelines, and local remuneration policies. The molecular tumor board plays a central role in this complex process. It consists of a multidisciplinary team of experts in the molecular and clinical aspects of cancer, including oncologists, pathologists, radiologists, geneticists, bioinformaticians, clinical scientists, and other specialists. Molecular tumor boards often follow a hybrid approach, mixing actual and virtual attendance, such that region hospitals can link with expert centers to discuss clinical cases.

To date, there are no standardized criteria for the operation of a molecular tumor board; however, in general terms, an ideal molecular tumor board would have oversight of the following areas:

1. Integration of the tumor molecular profile with other clinical features, such as tumor type and clinical presentation, to ensure that the molecular features are consistent with the pathologic diagnosis and to highlight genomic alterations that bear diagnostic or prognostic significance;
2. Identification of driver mutations and other molecular features of the tumor (such as genomic signatures) that may be targeted by standard-of-care or experimental therapies; and
3. Provision of a platform for discussing difficult cases and making informed decisions about treatment options.

IMPLEMENTATION OF PRECISION ONCOLOGY: CURRENT CHALLENGES

A key challenge in the field of precision oncology is access to genomic profiling and, in cases where a therapeutic target is identified, access to innovative treatment options. Testing without access to treatment options is futile, and failure to identify patients who carry a biomarker prevents the use of targeted therapeutics.

Although NGS ultimately might become an integral part of the management of patients with cancer, it will be key in the meantime to define which patients should receive comprehensive genomic

TABLE 7 Clinical trial databases.

Resource	Notes
ClinicalTrials.gov	Comprehensive database of clinical trials sponsored by the US National Institutes of Health; ability to search for trials is by disease type, drug name, or molecular marker
Cancer Research Institute Clinical Trial Finder	Cancer immunotherapy clinical trials, including molecularly guided studies
American Society of Clinical Oncology (ASCO) Clinical Trial Finder	Clinical trials listed by disease type, treatment, and location
National Cancer Institute (NCI) Clinical Trials Search	NCI-sponsored trials, including molecularly guided studies
European Union Clinical Trials Register	Clinical trials listed by disease type, intervention, and molecular target

profiling and at what stage of their disease. In this setting, reimbursement/coverage of comprehensive molecular profiling remains a pressing question. Whereas technological advances are constantly driving costs down, tumor testing can still cost a few thousand dollars. In many countries in which comprehensive molecular profiling is available in principle, reimbursement issues or the need for out-of-pocket spending will hamper the uptake of testing on a broader scale.[104,105] Accordingly, clear recommendations regarding which test (NGS vs. PCR/IHC/FISH) is preferred in each clinical situation will be critical to help guide clinicians through the ever more complex landscape of molecularly guided therapy options.

With the increased use of NGS testing, the need for accessible clinical expertise in precision oncology will rise. As outlined above, molecular tumor boards and peer-to-peer consultation can help in this setting, and the concept of virtual molecular tumor boards can help to serve communities and caregivers that otherwise would not have access to dedicated precision oncology programs.[106–109]

To offer the concept of precision oncology to a wider range of patients, health care systems will need to find sustainable ways to offer access to quality-assured molecular testing. Offering patients access to molecular profiling, regardless of their place of residence, is a considerable logistical undertaking. Building integrative networks that share centralized or decentralized testing facilities will allow more patients access to testing while leading to larger case volume, allowing for more cost-effective testing and mitigation of investment costs.

Finally, the principle of precision oncology will need to be integrated into physicians' training, with basic knowledge being taught in medical school and a focus on the path toward specialization. However, the need for training and building expertise does not just concern the direct caregiver level but also includes (molecular) pathologists, molecular biologists, scientists, bioinformaticians, and technical staff. To offer the whole spectrum of precision oncology, from testing to an individualized treatment plan in the form of an integrated health care solution, all experts contributing to the value chain should be formally trained to fulfill their given role. With the growing need for experts comes the great potential to grow and diversify the precision oncology workforce beyond (a few) privileged centers.

RACIAL DISPARITIES AND NEED FOR DATA DIVERSITY

An essential question in genomic research is the applicability of findings across diverse global populations and ethnicities. Variations in tumor biology and therapeutic responses among different populations are increasingly evident, necessitating a broader representation in research. Specific clinical behaviors, like increased cancer risk and aggressive tumor biology, are observed in certain ethnic groups because of shared genetic backgrounds.[110–112] For instance, African American men exhibit a higher prevalence and worse outcomes in prostate cancer,[113] underscoring significant racial disparities.[114] In this light, a recent study identified considerable molecular differences in the molecular profile across six major cancers when comparing patients of African ancestry versus patients of other ancestry.[112]

The predominant inclusion of high-income, predominantly White populations in genomic studies has led to skewed data, affecting the accuracy of clinical tools and diagnostic yields in non-White populations.[115] This disparity necessitates a deliberate effort to enhance the diversity in genomic research, encompassing various racial, socioeconomic, and geographic segments.

Barriers, such as lack of information, eligibility criteria, and health disparities, hinder the inclusivity in research participation. Initiatives like the Cancer Disparities Research Network (CDRN) and the Polyethnic-1000 (P-1000) are instrumental in addressing these barriers, aiming to facilitate the integration of diverse populations in genomic studies, thus fostering innovations and precision medicine approaches in oncology.

Moving forward, a reinforced commitment from institutions and researchers is imperative to drive a paradigm shift toward a more inclusive and representative genomic research landscape, enabling a deeper understanding of the intricate interplay between ancestry, ethnicity, and cancer-related outcomes.

SUMMARY AND CONCLUSIONS

Recent rapid advances in cancer genomics are revolutionizing the management of cancer, making it essential for health care professionals to understand the implications of genomic alterations and molecular subtyping for diagnosis, prognosis, and treatment selection. This article, based on a comprehensive review of the role of genomics in cancer care and on our multidisciplinary expertise in the field of precision oncology, represents a unique educational resource for clinicians, empowering them with the necessary tools to efficiently incorporate cancer genomics into their clinical practice. It is hoped that the widespread uptake of biomarker-informed precision oncology into routine clinical practice will ultimately improve outcomes for those living with cancer.

CONFLICT OF INTEREST STATEMENT

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