Comprehensive Genomic Profiling in Predictive Testing of Cancer

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Summary

Despite the rapid progress in the field of personalized medicine and the efforts to apply specific treatment strategies to patients based on the presence of pathogenic variants in one, two, or three genes, patient response to the treatment in terms of positive benefit and overall survival remains heterogeneous. However, advances in sequencing and bioinformatics technologies have facilitated the simultaneous examination of somatic variants in tens to thousands of genes in tumor tissue, enabling the determination of personalized management based on the patient's comprehensive genomic profile (CGP). CGP has the potential to enhance clinical decision-making and personalize innovative treatments for individual patients, by providing oncologists with a more comprehensive molecular characterization of tumors. This study aimed to highlight the utility of CGP in routine clinical practice. Here we present three patient cases with various advanced cancer indicated for CGP analysis using a combination of SOPHiA Solid Tumor Solution (STS, 42 genes) for DNA and SOPHiA RNAtarget Oncology Solution (ROS, 45 genes and 17 gene fusions with any random partners) for RNA. We were able to identify actionable genomic alterations in all three cases, thereby presenting valuable information for future management of these patients. This approach has the potential to transform clinical practice and greatly improve patient outcomes in the field of oncology.

Key words

Comprehensive genomic profile • Personalized medicine

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Introduction

Personalized medicine (PM) is a medical model of care which allows for the customization of health care, therapy, and diagnostic procedures taking into account the molecular basis of the disease. This model often uses laboratory testing focused on the selection of appropriate and optimal therapies based on comprehensive molecular and genetic analysis. One of the most important aspects of PM in the field of oncology is the identification of suitable molecular biomarkers that can be used for the estimation of prognosis and prediction of targets for innovative biological therapy [1-3].

Information about the molecular characteristics of individual tumors helps oncologists supplement traditional methods such as tissue localization and tumor histology. PM is in current clinical practice predominantly used in the form of single gene-based assays. However, this approach is being gradually substituted by next-generation sequencing (NGS) [4]. Analysis of tumor DNA using NGS allows for the creation of a comprehensive genomic profile (CGP) through the simultaneous evaluation of tens to thousands of genes at once. The use of a comprehensive gene panel has several advantages over analyzing individual genes as it enables simultaneous detection of all classes of genomic alterations known to drive malignant growth such as single nucleotide variants (SNV), insertions and

deletions (indels), copy number variations (CNV), gene rearrangements and fusions [5]. Additionally, there are also more complex biomarkers called genomic signatures, which include microsatellite instability (MSI status) and tumor mutational burden (TMB), that hold potential as valuable instruments in the immunotherapy of cancer patients [6,7].

The integration of PM and biomarker-guided therapy has instigated a paradigm shift in oncology, necessitating the adoption of CGP in routine clinical practice. Furthermore, CGP also offers a more efficient approach by utilizing often small amounts of available biopsy tissue, detecting gene fusions with unknown partners, helping to identify carcinomas of unknown primary, and facilitating the differential diagnosis of various tumors [8-10]. This approach provides oncologists with a comprehensive genomic profile of a patient's tumor, enhancing their ability to identify targeted therapies that offer a higher likelihood of success with fewer adverse effects.

This trend was recently acknowledged by the European Society for Medical Oncology (ESMO) in their 2020 recommendations regarding the use of NGS for identifying biomarkers in patients with metastatic cancer [11]. ESMO recommends the use of NGS for patients with various types of cancer including non-small cell lung cancer (NSCLC), prostate cancer, ovarian cancer, and cholangiocarcinoma. Additionally, NGS is recommended for determining TMB in patients with cervical cancer, salivary gland cancer, thyroid cancer, vulvar cancer, and neuroendocrine tumors. In the case of colorectal cancer, NGS may serve as a cost-effective alternative to PCR-based tests. ESMO also recommends and emphasizes the significance of multigene sequencing for clinical facilities as part of streamlining drug research and development [11]. The American Society of Clinical Oncology (ASCO) similarly recommends the use of NGS for identifying biomarkers in all patients whose cancer has at least one specific genetic alteration with regulatory approval for the use or exclusion of a particular drug. ASCO emphasizes the importance of genomic testing for all tumors, with an increasing number of tissue-agnostic drugs approved for cancers with high TMB, microsatellite instability-high (MSI-H), or neurotrophic tropomyosin receptor kinase (*NTRK*) fusions [12]. Therefore this research aimed to emphasize the importance and applicability of CGP in modern clinical practice.

Methods

Study design

During the period between 1. 9. 2020 to 18. 5. 2023, we conducted a thorough analysis of a cohort comprising 295 patients who were referred for CGP testing to determine their future treatment, prognosis, and further management as part of their personalized care. For the purpose of this study, we selected three unique and interesting cases. Molecular testing was performed at the Department of Medical Genetics of St. Elizabeth Cancer Institute in Bratislava as part of the standard clinical practice. All experiments conformed to the Helsinki Declaration, and all patients provided written informed consent.

Nucleic acid extraction

Nucleic acids required for analysis were extracted from tumor tissue in the form of formalin-fixed paraffin-embedded (FFPE) sections obtained following surgical resection of the tumor. All FFPE samples were reviewed by a pathologist to identify tumor-rich regions. Commercially available FFPE tissue kits – Maxwell® FFPE Plus DNA Kit (Promega) and Maxwell® RSC RNA FFPE Kit (Promega) – were used for nucleic acid isolation. The isolation was performed using the Maxwell® RSC Instrument (Promega) which employs paramagnetic beads for efficient isolation. The concentration of nucleic acids was determined using a Quantus fluorometer (Promega), with a concentration of nucleic acids ≥40 ng/µl being deemed suitable for further analysis.

Comprehensive genomic profiling

CGP analysis was in this study performed by a combination of two multi-gene NGS panels – SOPHiA Solid Tumor Solution (STS) and SOPHiA RNAtarget Oncology Solution (ROS). Both are hybridization capture-based NGS assays targeting genomic alteration involved in the most common solid tumors. STS targets 42 genes in DNA, 6 unique loci to detect MSI status, and gene amplification events in 24 genes. ROS covers transcripts of 45 genes at the RNA level and 17 RNA fusion genes in combination with any random fusion partner. Even though there is a 30-gene overlap between the two panels, we use the ROS gene panel in addition to the STS panel mainly due to its capability of detecting RNA gene fusions with any unknown partner.

To prepare DNA and RNA libraries from fixed

FFPE tissue, we utilized the SOPHiA Solid Tumor Solution and SOPHiA RNAtarget Oncology Solution, following the manufacturer's instructions. Firstly, we transcribed RNA into cDNA using random hexamers. Subsequently, we enzymatically fragmented the DNA into 90 to 250 bp, repaired them by adding adenine to the 3' end, and labeled them with adapter molecules. Then, we purified libraries of unligated adapters and added universal index sequences to the fragments using universal index primers (UP1-12) to distinguish the samples in the subsequent multiplex sequencing reaction during demultiplexing. Following this, we hybridized overnight a set of oligonucleotide sequences targeting genomic alterations of interest to the libraries prepared in the previous steps. After hybridization, we employed streptavidin-coated magnetic beads to capture the hybridized probes firmly bound to the targeted sequences of DNA and RNA libraries. We washed the beads twice to remove unhybridized nonspecific sequences and eluted the enriched library from the magnetic beads. We repeated the hybridization, capture, and enrichment steps once more to ensure maximum specificity. Subsequently, we amplified and purified the enriched libraries with cleaning beads. Following this step, we quantified the libraries using fluorescence measurement, determined library quality and fragment size using a 2100 Bioanalyzer (Agilent), and normalized the libraries to ensure uniform representation during the analysis of multiple samples simultaneously. Finally, we denatured and diluted the libraries to the final sequencing concentration and sequenced them on a NextSeq 550 analyzer (Illumina).

Bioinformatic data analysis

The data obtained in FASTQ format were analyzed using CGW SOPHiA DDM software (Sophia Genetics).

Classification of the detected variants

The detected variants were described according to HGVS (Human Genome Variation Society) [13]. The pathogenicity of the variants was classified based on valid ACMG criteria [14], and the clinical significance was defined according to TIER criteria [15]. The interpretation of variants is based on data published in databases and scientific publications and is conducted following current ACMG and TIER criteria. The results do not include benign variants (Class 1), likely benign variants (Class 2), and variants with unknown significance (Class 3) according to the ACMG criteria. Likewise, variants with unknown clinical significance (Class III) and benign/likely benign variants (Class IV) according to the TIER criteria (Fig. 1) are not included in the results but are part of the laboratory protocol. The results are valid only within the limits of the detection methods used.

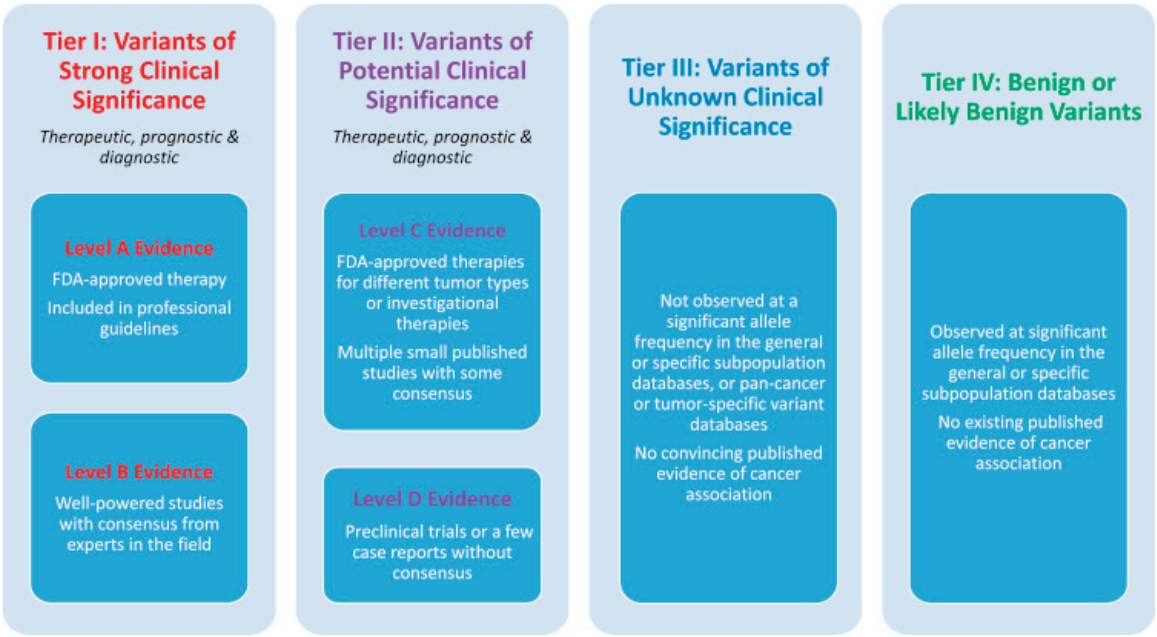

Fig. 1. Categorization of somatic variants based on their clinical significance. The figure presents an evidence-based categorization of somatic variants according to their clinical significance in cancer diagnosis, prognosis, and therapeutics. The classification system is structured into four tiers, each indicating different levels of clinical importance. Variants in tier I are of the strongest clinical significance, and variants in tier IV are benign or likely benign variants [15].

Results

As part of personalized care, patients referred for comprehensive genomic profiling (CGP) testing underwent a molecular genetic analysis using a panel of genes to aid in determining their future treatment, prognosis, and management. For this study, we identified and analyzed three intriguing case studies, including rare cases. The cohort consisted of patients with a malignant brain tumor, axillary metastases with the suspected origin of high-grade serous ovarian carcinoma, and unspecified fibromatosis.

Case 1

A 69-year-old male patient with an unspecified malignant brain tumor was referred for CGP analysis with the primary objective of identifying potential treatment options and optimizing his future clinical management. NGS of DNA and RNA obtained from tumor tissue was performed to identify possible actionable genomic alterations.

In the sample of DNA isolated from tumor tissue, we detected the presence of a somatic variant c.-146C>T in the *TERT* gene. The variant has unknown significance according to internationally accepted criteria (ACMG) and a potential clinical significance of IID (TIER) (Table 1). The somatic variant c.-146C>T (sometimes referred to as C250T) identified in the DNA sample is located in the promoter region of the *TERT* gene and was present in the tumor DNA sample at a frequency of 19.5 %. Interestingly, we also concurrently identified the presence of *FGFR3-TACC3* (F3T3) gene fusion in the RNA sample extracted from the tumor tissue. Applying the internationally accepted ACMG criteria, we classified this genomic alteration as pathogenic. According to the TIER criteria, we assigned this variant a classification of having potential clinical significance (IIC). Furthermore, the sample was MSS (microsatellite stable) and no somatic CNVs were detected.

Table 1. Detected somatic variant in the DNA sample of tumor tissue (case 1).

Gene	LRG sequence	Reference sequence	HGVS nomenclature	Depth	VAF	TIER classification	ACMG classification
TERT	LRG_343t1	NM_198253.2	c.-146C>T p.(C250T)	1681	19.5 %	IID	Variant of unknown significance

LRG, Locus Reference Genomic; HGVS, Human Genome Variation Society; VAF, Variant allele frequency.

Case 2

A 62-year-old female patient presented with axillary metastases and suspected high-grade serous ovarian carcinoma (HGSOC). The primary objective of the patient's referral for CGP analysis was to assess potential treatment options with TRK inhibitors or PARP inhibitors, based on the identification of *NTRK* fusions and any potential pathogenic variants within the *BRCA1/BRCA2* genes respectively. NGS of DNA and RNA obtained from the patient's tumor tissue was performed to identify actionable genomic alterations.

In the sample of DNA isolated from tumor tissue, we were able to detect somatic pathogenic variant c.595G>T in the *TP53* gene according to internationally accepted criteria (ACMG) and with a potential clinical significance of IIC (TIER). The somatic variant c.595G>T analyzed from the DNA sample of tumor tissue is located in exon 6 of the *TP53* gene. This variant was present in the tumor DNA sample at a frequency of 9.4 %. Due to its presence, a reading frame shift occurs, and a premature STOP codon is incorporated, which is likely to lead to the formation of a non-functional or truncated protein. The variant is classified as pathogenic in the ClinVar database and was detected in lung, breast, gastrointestinal, pancreatic, and esophageal cancers in the somatic variant database Cosmic. Classification software, such as Varsome and Franklin, classify it as pathogenic with potential clinical significance. Based on internationally accepted ACMG criteria, we classify this variant as pathogenic. According to TIER criteria, we classify variant c.595G>T as a variant with potential clinical significance (IIC).

In the sample of RNA isolated from tumor tissue, we were able to detect the same variant (c.595G>T in the *TP53* gene) and also another somatic pathogenic variant c.3018_3021del in the *BRCA1* gene. The somatic

variant c.3018_3021del is located in exon 11 of the *BRCA1* gene. This variant was present in the tumor RNA sample at a frequency of 58.0 % (Table 2). Due to its presence, a reading frame shift occurs, and a premature STOP codon is incorporated, which is likely to lead to the formation of a non-functional or truncated protein. The variant is classified as pathogenic in the ClinVar database. Classification software, such as Varsome and Franklin, classify it as pathogenic with strong clinical significance. Based on internationally accepted ACMG criteria, we classify this variant as pathogenic. According to TIER criteria, we classify variant c.3018_3021 as a variant with strong clinical significance (IA). The analyzed sample was MSS and no somatic CNVs were detected.

Table 2. Detected somatic variants in samples of tumor tissue isolated from DNA or RNA (case 2).

Gene (DNA/RNA)	LRG sequence	Reference sequence	HGVS nomenclature	Depth	VAF	TIER classification	ACMG classification
***TP53* (DNA)**	LRG_321	NM_000546	c.595G>T p.(Gly199*)	3318	9.4 %	IIC	Pathogenic variant
***TP53* (RNA)**	LRG_321	NM_000546	c.595G>T p.(Gly199*)	4359	9.1 %	IIC	Pathogenic variant
***BRCA1* (RNA)**	LRG_292	NM_007294	c.3018_3021del p.(His1006Glnfs*17)	1205	58.0 %	IA	Pathogenic variant

LRG, Locus Reference Genomic; HGVS, Human Genome Variation Society; VAF, Variant allele frequency.

Case 3

A 61-year-old female patient with unspecified fibromatosis in the thigh region was referred for CGP analysis with the primary objective of this analysis being evaluation of the *CTNNB1* gene for differential diagnosis of desmoid tumors. NGS of DNA and RNA obtained from tumor tissue was performed to identify possible actionable genomic alterations.

In the sample of DNA and RNA isolated from tumor tissue, we were able to detect likely pathogenic somatic variant c.133T>C in the *CTNNB1* gene according to internationally accepted criteria (ACMG) with a potential clinical significance of IIC (TIER). The somatic variant c.133T>C identified from the DNA sample of tumor tissue is located in exon 3 of the *CTNNB1* gene. This variant was present in the tumor DNA sample at a frequency of 20.8 % (Table 3). The variant c.133T>C is described in the ClinVar database as pathogenic (3×) and as likely pathogenic (11×). It is also described in the somatic variant database Cosmic in patients with liver carcinoma, soft tissue tumors, adrenal gland, kidney, and colon cancer. The classification software Varsome classifies it as a likely pathogenic variant with potential clinical significance (IIC). According to TIER criteria, we classify the variant as having potential clinical significance (IIC). The examined sample was MSS and no somatic CNVs were identified.

Table 3. Detected somatic variant in the DNA sample of tumor tissue (case 3).

Gene	LRG sequence	Reference sequence	HGVS nomenclature	Depth	VAF	TIER classification	ACMG classification
CTNNB1	LRG_1108t1	NM_001904.4	c.133T>C p.(S45P)	4300	20.8 %	IIC	Likely pathogenic variant

LRG, Locus Reference Genomic; HGVS, Human Genome Variation Society; VAF, Variant allele frequency.

Discussion

Personalized medicine based on patients' actionable genomic alterations is a promising and rapidly advancing area in the field of modern oncology. The development of targeted biological drugs tailored against specific mutations in tumors is slowly replacing nonselective conventional standard therapies. The proven clinical benefits associated with certain specific targeted therapies, the rapidly increasing number of actionable biomarkers, and the declining cost of comprehensive genomic profiling (CGP) have resulted in a greater demand for these types of assays.

In this study, we describe our experience with the clinical use of CGP through three patient case studies. The first case involved the identification of a somatic variant c.-146C>T in the *TERT* gene (chromosome 5) promoter, a hotspot mutation commonly found in oligodendro-gliomas with 1p and 19q deletions, as well as in most glioblastomas. In the scientific literature, the occurrence of this variant has been described in patients with brain cancer (among others, also in patients with thyroid cancer and bladder cancer) [16-18]. In diffuse infiltrating gliomas, the presence of this mutation, in the absence of *IDH* mutations, is associated with shorter overall patient survival [19]. Arita *et al.* demonstrated the clinical significance of testing the combination of *TERT* mutation and MGMT (O(6)-methylguanine-DNA methyltransferase) methylation status in glioblastoma patients. *TERT* mutated-MGMT unmethylated tumors were associated with the poorest prognosis, highlighting *TERT* as a potential therapeutic target. Integrating *TERT* mutation as an additional biomarker enhances diagnostic accuracy, prognostication, and treatment selection for glioma patients, particularly for those with *TERT*-mutated and MGMT-unmethylated glioblastoma who have limited response to standard treatments [20].

Furthermore, we identified a *FGFR3-TACC3* (F3T3) gene fusion in this patient through the RNA portion of the CGP analysis. This gene fusion is listed in the ChimerDB fusion gene database and has been previously reported in patients with brain cancer, as well as in patients with bladder cancer and non-small cell lung cancer. The same fusion was identified in a 2020 study by Di Stefano *et al.* [21], which showed that patients with *IDH* wild-type glioblastoma and the F3T3 fusion had better response and overall survival with standard radiochemotherapy than patients without the fusion. The study conducted by Di Stefano *et al.* offers a thorough characterization of F3T3-positive gliomas, highlighting their unique molecular, radiological, and clinical attributes in comparison to *IDH* wild type gliomas. The results of their findings suggest that F3T3-positive gliomas should be recognized as a distinct subgroup of brain tumors, warranting specialized approaches for accurate diagnosis, prognostication, and therapeutic interventions. Patients with metastatic urothelial carcinoma and *FGFR3* alterations may also benefit from treatment with *FGFR* tyrosine kinase inhibitors (Erdafitinib) after progression on platinum-based treatment [22]. The literature even describes a very specific case of a patient with papillary glioneuronal tumor (*IDH* wild-type, F3T3 fusion, *TERT* promoter mutation c.-124C>T (C228T)), who was treated with *FGFR* tyrosine kinase inhibitor (Erdafitinib) after disease progression following the third resection, but without observed response to the treatment [23]. F3T3 fusions demonstrate increased sensitivity to *FGFR*-targeted therapies compared to other *FGFR* aberrations. This finding carries clinical significance, as patients with aggressive tumors such as glioblastoma and bladder cancer, where T3F3 fusions have been identified, have limited treatment options. Although F3T3 fusions are rare, they are present in a wide range of solid tumor types, emphasizing the importance of including their analysis in screening procedures for *FGFR*-targeted trials in solid tumors [24]. CGP analysis of this patient's tumor identified important genomic alterations with potential for prognosis and use in future patient management.

In the case of a second patient with axillary metastases with suspected HGSOC origin, we conducted CGP to determine potential treatment options with TRK or PARP inhibitors based on the identification of *NTRK* fusions and any potential pathogenic variants within the *BRCA1/BRCA2* genes respectively. In the sample of DNA and RNA isolated from tumor tissue, we were able to detect a c.595G>T variant in the *TP53* gene. This variant is located in the DNA binding domain where approximately 80 % of all identified pathogenic *TP53* variants are located, which is important for binding to *AXIN1* [25]. *TP53* is the most frequently mutated gene in human cancer, with a prevalence of 40-50 % across various cancer types. In HGSOC, *TP53* mutations are even more prevalent, occurring in approximately 95 % of cases [26]. Wong *et al.* demonstrated that patients with *TP53* wild-type HGSOC had poorer survival and increased chemoresistance compared to those with *TP53* mutations [27]. Conversely, Ghezelayagh *et al.* found no overall survival benefit associated with *TP53* mutations in HGSOC, but these mutations were linked to increased sensitivity to platinum-based chemotherapy [28]. However, arguably more important was the identification of another somatic variant

c.3018_3021del in the *BRCA1* gene. This variant was present in the tumor RNA sample at a frequency of 58.0 % which suggests the possibility that it could be a germline variant, and we recommend considering verification of its germline status by analyzing DNA from peripheral blood [29]. Patients with HGSOC, prostate, pancreatic, triple-negative breast cancer, and with pathogenic variants in the *BRCA1* gene may benefit from treatment with PARP inhibitors.

The third presented case was a 61-year-old female patient with unspecified fibromatosis who was referred for CGP analysis with the primary objective of this analysis being evaluation of the *CTNNB1* gene for differential diagnosis of desmoid tumors. The differential diagnosis of fibromatosis and desmoid tumors can be challenging because they are similar in their clinical presentation and histological features. However, there are some key differences that can aid in the diagnosis. Desmoid fibromatosis is a locally aggressive neoplasm that affects young to middle-aged adults and occasionally children. It can occur sporadically or as part of familial adenomatous polyposis (FAP) syndrome. The activation of the canonical Wnt/beta-catenin signaling pathway plays a key role in both situations. In sporadic cases, mutations occur in *CTNNB1* with the most common types being point mutations involving phosphorylation sites encoded by exon 3, while in FAP, germline mutations occur in the *APC* tumor suppressor gene. Both mutations result in the accumulation of non-phosphorylated beta-catenin in the cytoplasm and its translocation to the nucleus, activating the transcription of genes that promote proliferation and increased cell survival [30]. In the sample of DNA and RNA isolated from tumor tissue, we were able to detect somatic variant c.133T>C located in exon 3 of the *CTNNB1* gene. Approximately 85 % of patients with desmoid tumors have been identified with the presence of a somatic pathogenic (or likely pathogenic) variant in the *CTNNB1* gene, with the c.133T>C variant occurring at a frequency of 8 % [31]. Desmoid tumors present a highly variable prognosis, characterized by an unpredictable disease course. Notably, approximately 20-30 % of patients experience spontaneous regressions within a 2 to 3-year monitoring period. Following an initial growth phase, these tumors often stabilize. However, it is important to acknowledge the high recurrence rate associated with desmoid tumors, which significantly diminishes the effectiveness of surgical intervention. Given the uncertain disease trajectory and the potential for spontaneous regressions, the current preferred management approach for patients with desmoid tumors is active surveillance, prioritizing close monitoring rather than immediate surgical intervention [32]. Furthermore, oral administration of Vinorelbine is an effective and well-tolerated therapy in patients with advanced or progressive desmoid fibromatosis, with prolonged activity observed in patients with tumors carrying the c.134C>T or c.133T>C variants in the *CTNNB1* gene [33].

CGP has emerged as a potent tool that provides oncologists with a more comprehensive molecular characterization of tumors, enabling enhanced clinical decision-making and the personalization of innovative biological therapies for individual patients. Nevertheless, despite its potential benefits, CGP still presents challenges that require careful consideration. One such challenge pertains to the high initial cost associated with acquiring an NGS-capable genetic analyzer. Additionally, accurate classification of somatic variants necessitates the high expertise of molecular biologists and geneticists and requires competent personnel. In addition, it is essential to conduct further research to determine the optimal panel size for routine clinical practice. While complex genomic signatures such as TMB hold great promise in the field of personalized oncology, it necessitates the analysis of hundreds of genes. Therefore, it is crucial to consider, whether the potential benefits of analyzing these complex biomarkers outweigh the costs and practicality of using smaller gene panels in routine clinical practice. Furthermore, it is crucial to evaluate whether universal testing or targeted testing of specific patient groups would deliver the greatest benefit. This approach has the potential to transform clinical practice and greatly improve patient outcomes in the field of clinical oncology.

Conclusions

Comprehensive genomic profiling (CGP) in personalized medicine holds promise to improve clinical decision-making and enhance treatment outcomes for cancer patients. This study highlights three cases where CGP analysis successfully identified actionable genomic alterations, demonstrating the transformative impact of this approach on clinical practice and patient management in oncology.

Conflict of Interest

There is no conflict of interest.

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