Original Article

Clinical Utility of Large Next-Generation Sequencing Panel Across Diverse Tumour Types: A Single-Center Retrospective Analysis

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Received: 11-Oct-2023;
Revision: 17-Dec-2024;
Accepted: 01-Feb-2025;
Published: 11-Apr-2025

ABSTRACT

Background: In complex real-world clinical practice, the application of advanced technologies, such as next-generation sequencing (NGS), is crucial for achieving the most reasonable and relatively effective therapy for cancer patients at each stage of treatment. **Aim:** This study aimed to retrospectively analyze the clinical utility of a large NGS panel in the management of solid tumors within a clinical practice setting. **Method:** A comprehensive NGS panel was used to detect diagnostic, prognostic, and therapeutic biomarkers in solid tumors on a commercially available platform. NGS assay was performed on 431 specimens from 416 patients. **Results:** At least 1 actionable variant was in 89.1% of all specimens. The most frequently altered gene was TP53, followed by EGFR, KRAS, PIKC3A, and RB1. In 55.7% of cases, at least 1 therapeutically targetable variant was identified, including 25.3% of cases harboring variants for which a targeted therapy was available for the disease and 26.0% of cases harboring variants for which a targeted therapy was available for other diseases. The median tumor mutational burden (TMB) was 10.3 mutations/Mb. Microsatellite instability (MSI) was available for 352 patients, and only 6 cases were MSI-high. The patients receiving targeted therapy and/or immune checkpoint inhibitors survived significantly longer than those receiving chemotherapy and/or radiotherapy (P = 0.001). **Conclusion:** Our results demonstrate the significant clinical utility of comprehensive genomic profiling in the routine clinical testing of patients with solid tumors.

KEYWORDS: *Cancer, mutation, next-generation sequencing, precision medicine, target therapy*

INTRODUCTION

Cancer incidence and mortality rates are growing sharply with socioeconomic development and the advent of an aging society worldwide.[1] Cancer is one of the leading causes of death worldwide and is responsible for nearly 10 million cancer-related deaths

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_733_23

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How to cite this article: Chen HP, Guo ZY, Zhang LM, Sun S, Huang JX, Sha M, *et al.* Clinical utility of large next-generation sequencing panel across diverse tumour types: A single-center retrospective analysis. Niger J Clin Pract 2025;28:352-9.

in 2020.[1] In China, cancer is the leading cause of death, with approximately 3 million cancer-related deaths recorded in 2020.[1,2] Furthermore, the incidence and mortality of cancer will continue to rise in China with an increasing trend of population aging and the widespread existence of unhealthy lifestyles. Although different cancers have different causes and symptoms, they occur because of abnormalities in the deoxyribonucleic acid (DNA) sequence of the genomes of cancer cells. In the meantime, targeted therapies acting on specific molecular targets have become one of the mainstream cancer treatments, while molecular testing has become the cornerstone of precise oncology. The detection of somatic mutations in cancer is of great significance for guiding targeted therapy, monitoring drug resistance, and predicting prognosis.

The widespread application of molecular testing, which is used to evaluate all potentially actionable targets and diagnostic, prognostic, and predictive biomarkers, makes it more complex with the increasing availability of lifesaving targeted therapies for cancer patients. Various methods can be used to detect somatic variants in cancer specimens, such as next-generation sequencing (NGS), droplet digital polymerase chain reaction (ddPCR), and amplification-refractory mutation system (ARMS), which have their advantages and disadvantages.[3,4] The main disadvantage of traditional companion diagnosis is that one biomarker usually corresponds to only one drug per cancer. Therefore, traditional molecular testing will not be able to screen all targeted drugs that patients may benefit from in a single test. With the increasing availability of targeted drugs, molecular testing has advanced from single-gene approaches to multigene parallel testing methods. On the other hand, NGS can detect dozens and hundreds of genes or even whole genomes at a time, comprehensively screen all potential targeted therapies, and cover predictive biomarkers for immune checkpoint inhibitors (ICIs) such as tumor mutational burden (TMB) and microsatellite instability (MSI).[5] Several NGS panels, including OncomineDx Target Test (46 genes), MSK-IMPACT (468 genes), and FoundationOne Liquid CDx (324 genes), have been approved by the U.S. Food and Drug Administration (FDA), whereas NGS panels approved by the Chinese National Medical Products Administration (NMPA) were small panels. To date, NGS has been widely used in clinical practice because of its ultrahigh throughput, high sensitivity, and cost reduction in recent years. Larger NGS panel-based molecular testing is becoming more common in clinical cancer management.[6] In this study, we retrospectively analyzed 416 cancer patients at our hospital using a commercial NGS panel covering 800 genes (OutdoClinic).

Materials and Methods

Patients

A total of 416 patients with histologically confirmed cancer from Taizhou People's Hospital were included. All patients underwent an 800 gene NGS panel (OutdoClinic) assay and 12 patients underwent serial testing. Written informed consent was obtained from each patient for the use of peripheral blood and tissue samples for research purposes. The study was approved by the ethical committees of Taizhou People's Hospital (KY 2023-084-01).

NGS

NGS was performed using a commercial NGS panel covering 800 genes (OutdoClinic). Briefly, formalin-fixed paraffin-embedded (FFPE) tissues were deparaffinized with xylene and DNA was then extracted using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genomic DNA from leukocytes was extracted using a QIAamp DNA Midi kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Plasma was obtained by centrifuging whole blood at 1600 × g for 20 min at room temperature and then centrifuged at 16,000 × g for 10 min to remove residual cells and debris. Cell-free DNA (cfDNA) was extracted from the plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sequencing libraries were prepared using the SureSelect XT reagent (Agilent Technologies, CA, USA) and a KAPA Hyper Prep kit (KAPA Biosystems, MA, USA). Finally, 150 bp paired-end sequencing was performed with a mean coverage of 5000 × for FFPE samples and 10000 × for plasma samples on the Illumina NextSeq 500 (Illumina, CA, USA).

Bioinformatics analyses

Raw reads were aligned to the human genome reference sequence (GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA, v0.7.12). Polymerase chain reaction (PCR) duplicates were removed using Picard. Local realignment and base quality recalibration were performed using GATK (v4.2.0.0). Single-nucleotide variants (SNVs) and small insertions or deletions (INDELs) were subsequently called by MuTect2 (v4.1.4.1). The copy number variants (CNVs) were called by Contra (v2.0.8). TMB was calculated as the number of somatic coding alterations per megabase, including non-synonymous variants and INDELs. Samples with two or more altered microsatellite loci were classified as MSI-high (MSI-H), and samples with one altered microsatellite locus were classified as MSI-low (MSI-L), and otherwise microsatellite stability (MSS).

Statistical analyses

Data were analyzed using the SPSS software v25.0 (IBM SPSS, CA, USA). The differences in TMB between the groups were evaluated using the nonparametric Mann–Whitney–Wilcoxon test. Survival curves for overall survival (OS) were generated using the Kaplan–Meier method. The log-rank test was used to compare OS between groups. The hazard ratio (HR) was derived from the Cox regression model. A *P* value of less than 0.05 was considered statistically significant.

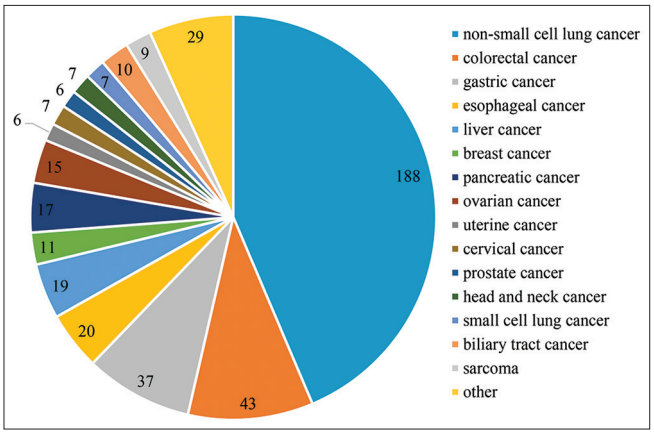

Figure 1: Tumor types

Results

Patient characteristics

We retrospectively analyzed 416 cancer patients who underwent NGS testing. Of these patients, 184 (42.7%) were female. The average age of the cancer patients was 62.7 years old (range, 25–90 years). A total of 431 clinical specimens were obtained, including tissues (n = 255, 59.2%), plasma (n = 167, 38.7%), pleural effusion (n = 8, 1.9%), and ascitic fluid (n = 1, 0.2%). All exfoliated cell and tissue specimens were combined in the following analysis because the pleural effusion and ascitic fluid specimens were too small. More than 29 types of tumors were included in the present study. As shown in Figure 1, the most common cancer

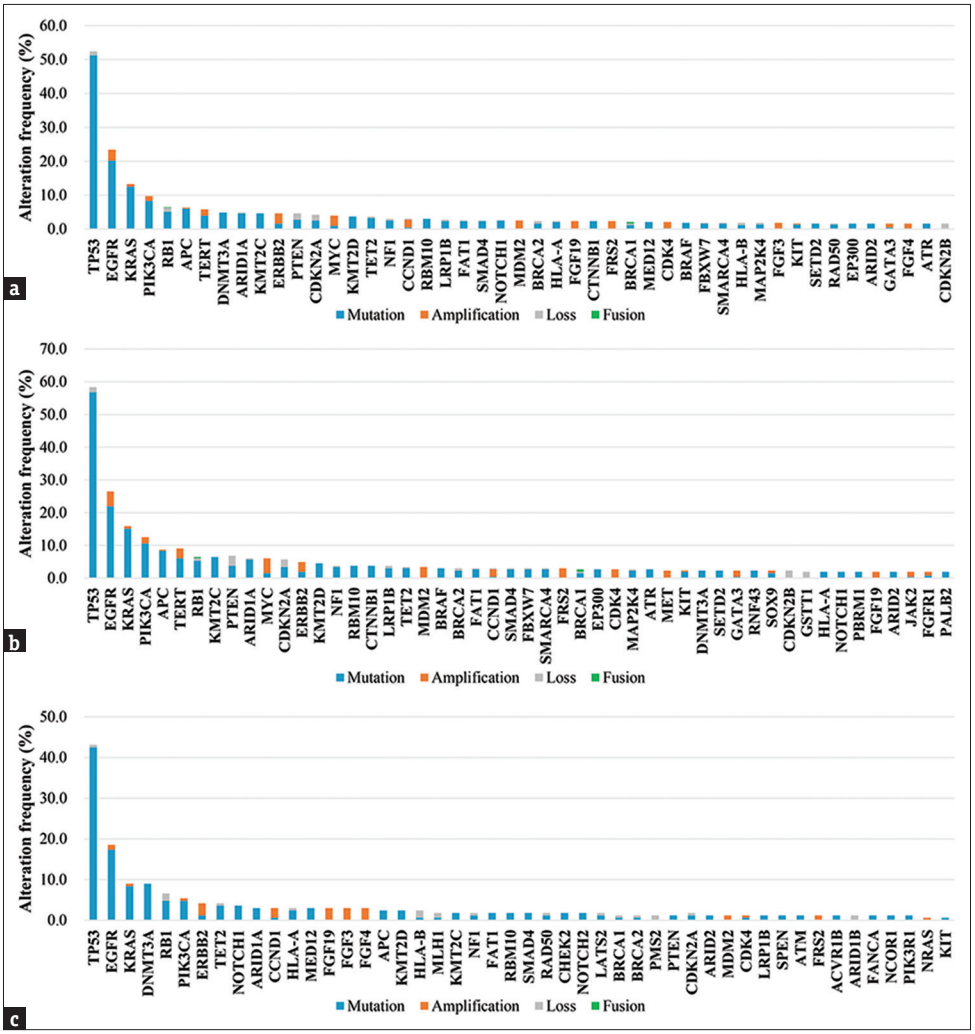

Figure 2: Most prevalent genomic alterations by gene. (a) a total of 431 cases. (b) tissues. (c) plasma

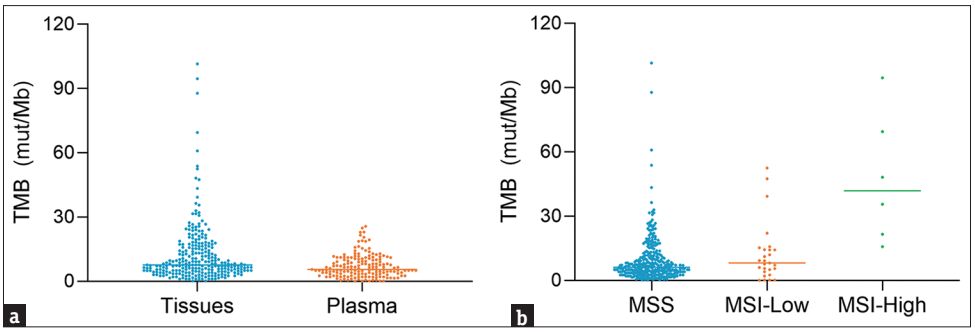

Figure 3: The relationship between MSI and TMB. (a) TMB by sample types. (b) TMB by MSI status

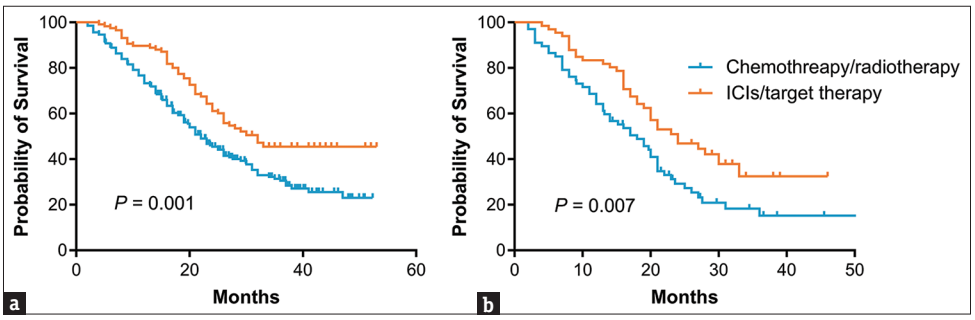

Figure 4: Kaplan-Meier plots of OS. (a) 323 patients. (b) 133 NSCLC patients

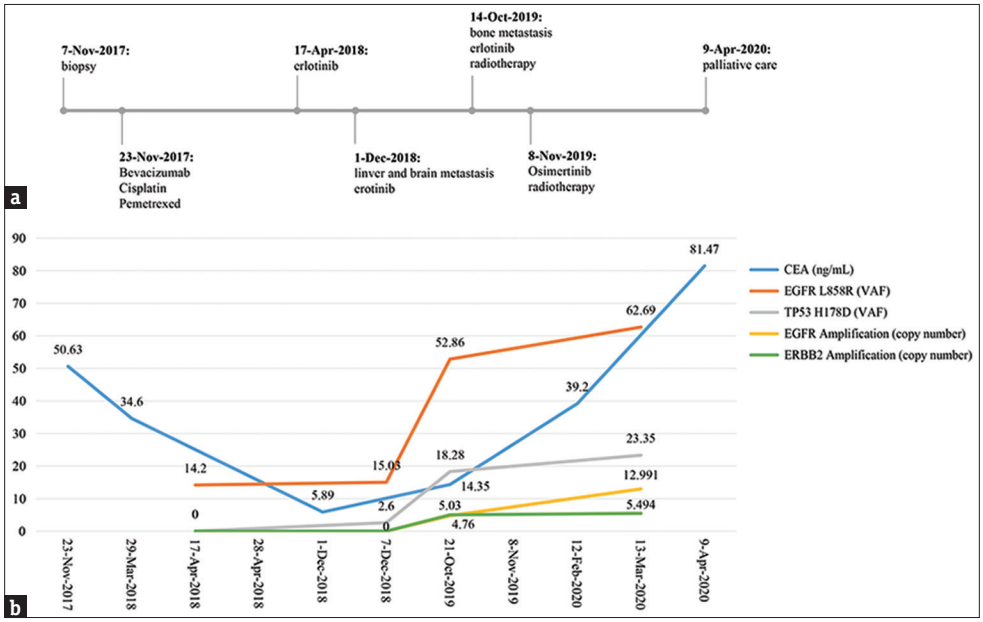

Figure 5: Clinical presentation. (a) timeline of disease course. (b) changes of serum CEA and variants in ctDNA of patient during treatments

type was non-small cell lung cancer (NSCLC; *n* = 188, 43.6%), followed by colorectal cancer (CRC; *n* = 43, 10.0%), gastric cancer (*n* = 37, 8.6%), esophageal cancer (*n* = 20, 4.6%), and liver cancer (*n* = 19, 4.4%).

Actionable variants

Twelve patients underwent at least two tests during the course of the disease. Overall, at least 1 actionable variant was in 89.1% (*n* = 384) of all specimens, with an average of 4.0 variants per sample (range 0–26), 96.6% (*n* = 255) of tissue specimens with an average of 5.0 variants per sample (range 0–26), and 77.2% (*n* = 126) of plasma specimens with an average of 3.0 variants per sample (range 1-14). The most frequently altered genes were TP53 (52.0%), followed by EGFR (20.9%), KRAS (13.2%), PIKC3A (9.7%), and RB1 (6.5%) [Figure 2]. In tissue specimens, the most frequently altered gene was TP53 (58.0%), followed

by EGFR (23.1%), KRAS (15.9%), PIKC3A (12.5%), and APC (8.7%). In plasma specimens, the most frequently altered gene was TP53 (42.5%), followed by EGFR (17.4%), DNMT3A (9.0%), KRAS (9.0%), and RB1 (6.6%).

In 51.3% (221/431) of cases, at least one therapeutically targetable variant was identified, including 25.3% (n = 109) of cases harboring variants for which a targeted therapy was available for the disease. Furthermore, 26.0% (n = 112) of cases harbored variants for which a targeted therapy was available for other diseases. Drug-resistant variants were identified in 32 (7.4%) patients. In addition, 55.8% (24/43) of CRC cases were RAS, BRAF, and ERBB2 wildtypes. Among these CRC patients, it was interesting that 2 patients harbored EML4-ALK and GOPC-ROS1 fusions, respectively. ALK fusion was found in 1 case, whereas no case harbored ROS1 fusion among 3839 CRC patients in cBioPortal. Both ALK and ROS1 fusions are rare in CRC.

TMB and MSI

The median TMB of tissue specimens (tTMB, n = 259) was 7.6 mutations/Mb (range, 0.1–101.4 mutations/Mb), and the median TMB of plasma specimens (pTMB, n = 93) was 5.9 mutations/Mb (range, 0–25.6 mutations/Mb) [Figure 3]. The tTMB was significantly higher than the pTMB (P < 0.001). MSI data were available for the 352 patients. MSI-H was detected only in the cancer tissues (n = 6). All MSI-H cases had high TMB, with a median TMB of 41.8 mutations/Mb (range, 15.8– 94.5 mutations/Mb). In the cancer tissues of patients with MSI-L (n = 13), the median tTMB was 14.4 mutations/Mb (range, 2.5–52.5 mutations/Mb). In patients with MSS in cancer tissues (n = 240), the median tTMB was 7.3 mutations/Mb (range, 0.1–101.4 mutations/Mb). There was a statistically significant difference in tTMB between distinct MSI status groups (P < 0.001). For plasma specimens, the median pTMB was 5.3 mutations/Mb (range, 0–11.2 mutations/Mb) and 3.6 mutations/Mb (range, 0.1–19.4 mutations/Mb) in MSI-L (n = 14) and MSS tumors (n = 79), respectively. There was no significant difference in the pTMB between the distinct MSI status groups (P = 0.736). When tTMB and pTMB were combined, there was a statistically significant difference in TMB between distinct MSI status groups (P = 0.001).

Clinical outcomes

Of the 416 cancer patients, 206 received chemotherapy and/or radiotherapy, and 117 received ICIs or targeted therapy. Ninety-three patients without information about the type of anticancer therapy or who did not receive anticancer treatment were excluded from the survival analysis. The median follow-up was 25.3 months [95% confidence interval (CI) 22.6–28.0], and the 4-year overall survival rate was 30%. Median overall survival (OS) for 117 patients receiving ICIs or targeted therapy was 32.0 months, which was significantly longer than that of those who received chemotherapy and/or radiotherapy (22.0 months, HR = 0.586, 95%CI: 0.427–0.804, P = 0.001, Figure 4). We performed further analysis on NSCLC (n = 133) because there was only a maximum of 38 cases of other types of cancer. NSCLC patients receiving ICIs or targeted therapy had a median OS (24.1 months) than those who received chemotherapy and/or radiotherapy (17.0 months, HR = 0.564, 95%CI: 0.368–0.864, P = 0.009).

Case presentations

Case 172 was a 77-year-old female never-smoker with a newly diagnosed advanced lung adenocarcinoma. Tumor volume decreased slightly after being treated with 5 cycles of bevacizumab, cisplatin, and pemetrexed. In April 2018, NGS analysis of her plasma revealed a somatic EGFR mutation (L858R) with a variant allele fraction (VAF) 14.2%) [Figure 5]. The patient was then treated with erlotinib (150 mg/day) and achieved progression-free survival (PFS) of 7.5 months according to circulating tumor DNA (ctDNA) status at baseline and follow-up. In December 2018, the serum level of carcinoembryonic antigen (CEA) returned to normal, whereas NGS analysis of ctDNA revealed a slightly increasing VAF of the EGFR p.L858R with the emergence of a new TP53 mutation (H178D) with a VAF of 2.6%. Computed tomography (CT) scan revealed small metastatic sites in the liver and brain. In October 2019, the patient complained of lower back pain and slightly limited activity for 4 months, and a subsequent CT scan revealed multiple bone metastases, including the iliac bone and vertebral body. A dramatic increase in VAF of EGFR L858R was found by ctDNA analysis, and the level of TP53 H178D also dramatically increased to 18.28%. Two new variants, ERBB2 amplification (5.03×) and EGFR amplification (4.76×), appeared. The patient received radiotherapy with a total dose of 40 gy/20 f for the bone lesions on November 8, 2019. The patient was then switched to osimertinib (80 mg/day), and the tumor continued to progress. Unsurprisingly, the ctDNA assay showed that the VAFs of EGFR L858R and TP53 H178D and the copy numbers of ERBB2 and EGFR continued to increase. The patient died 4 months later.

Case 307 was a 64-year-old male patient with newly diagnosed stage IIC colon cancer in February 2019. NGS assay showed that the tumor had an EML4-ALK fusion and was MSS and wild-type RAS and BRAF. The Patient received adjuvant chemotherapy with 6 cycles of capecitabine and oxaliplatin. To date, the patient has remained disease-free. Case 165 was a 78-year-old female who was diagnosed with stage IIIB colon cancer in June

2016 and received 8 cycles of mFOLFOX. The patient relapsed and underwent surgery in December 2019. NGS assay showed that the tumor had a GOPC-ROS1 fusion and was MSS and wild-type RAS and BRAF. The patient then received crizotinib, which was stopped after 4 months due to grade 3 vomiting. Serum carcinoembryonic antigen (CEA) level was elevated in March 2022, with a progression-free survival (PFS) of 27 months.

Discussion

Technological innovation has enriched the connotations of precision medicine. Precise diagnosis and treatment of cancer have continued to progress from intuitive to subtle and from "disease" to "gene". In complex real-world practice, the application of advanced technologies such as NGS and making good use of the "weapons" of precision treatment in the whole process of disease management is an important issue faced by clinicians. During the past decade, rapid advances in NGS and innovations in bioinformatics have outpaced its clinical application. This retrospective study evaluated the clinical utility of a large NGS panel in cancer patients. The most frequently altered genes were TP53, EGFR, KRAS, PIKC3A, and RB1. Therapeutically targetable variants were observed in 221/431 cases (51.3%) overall. The patients treated with ICIs and/or targeted therapy survived significantly longer than those treated with chemotherapy and/or radiotherapy.

As we all know, targeted therapy and immunotherapy, two new anti-tumor therapies, have been widely used in the treatment of many types of cancer, and are also the mainstream treatment schemes for these diseases. For targeted therapy and immunotherapy to develop precision medicine, evaluating the matching degree of anticancer drugs and the patient is an unavoidable prerequisite. NGS assays can help clinicians evaluate whether a patient is suitable for a certain therapeutic drug at the molecular level, and are also an important part of evaluating the effect of drugs in cancer patients. Large NGS panels can detect variants in hundreds of genes in a single sample at a time, which is very beneficial for detecting rare variants in key genes. It can provide patients with the opportunity to be treated with more approved drugs, participate in clinical trials when meeting the inclusion criteria, and allow more possibilities for disease cure. For advanced cancer, previous studies revealed a wide range of clinically actionable alterations in patients with advanced cancer, ranging from 40% to 94%, using comprehensive NGS panels.[7-9] In addition, approximately 25% of patients receive NGS-guided therapy. A large-scale, prospective study of 1,015 patients with advanced cancers by Cobain *et al*.[7] demonstrated the actual clinical value of NGS-based tumor companion diagnostics that improved the survival of patients, controlled the treatment costs, and avoided high expenses caused by delaying the disease. In the present study, therapeutically targetable variants were discovered in 221 patients (51.3%), including 109 cases harboring variants for which a targeted therapy was available for the disease. We identified two colon cancer patients with the EML4-ALK and GOPC-ROS1 fusions. The patient with EML4-ALK fusion and TNM stage IIC received chemotherapy and was disease-free at the last follow-up (43 months). CRC patients with ALK fusion may benefit from crizotinib and alectinib.[10] Another patient with GOPC-ROS1 fusion was treated with crizotinib and experienced recurrence after 27 months. The cancer was also MSS, as described previously in the literature.[11] CRC patients with ROS1 fusion may benefit from crizotinib or entrectinib.[10,11] Furthermore, a recent study by Tie *et al*.[12] revealed that two postoperative ctDNA assays could save nearly half of the patients with stage II colon cancer from adjuvant chemotherapy without compromising recurrence-free survival. This may change the level of ctDNA assays recommended by the guidelines, and even ctDNA assays may become one of the standard clinical testing methods. At the same time, ctDNA assay also provides a more sensitive detection method for tumor monitoring than imaging examination.[13] For cancers of unknown primary origin, a large NGS assay can provide a large amount of information for making treatment decisions.[7,14] However, the mechanisms underlying cancer occurrence and development are complex. Although a large number of cancer-related genes have been identified, the mechanisms of many genes in cancer are still unknown. Further studies are required to explore the specific molecular mechanisms involved in cancer development and progression. A large NGS panel can more comprehensively determine mutation profiles and accelerate the clinical translation of cutting-edge scientific research and its results. The light on NGS-based precision cancer medicine is shining into reality.

A general survey of cancer NGS assays approved by the U.S. FDA, including FoundationFocus™ CDxBRCA LOH, OncomineDx Target Test (46 genes), Praxis Extended RAS Panel (2 genes), MSK-IMPACT (468 genes), and FoundationOne Liquid CDx (324 genes) showed that the future development trend of cancer NGS assays must be from a single type of cancer to multiple types of cancers, a single gene to multiple genes, and a smaller panel to a larger panel. A large NGS panel usually contains hundreds of genes, including thousands of genes, which generally cover a wide range of cancer

types and more cancer-related genes and variant types, and provide more comprehensive clinical diagnosis, treatment suggestions, and curative effect evaluation. Although considered the gold standard for TMB measurement, whole exome sequencing (WES) remains confined to research settings owing to substantial costs, longer turnaround times, and large amounts of input DNA. Previous studies have demonstrated that TMB assessed by a large NGS panel has a higher agreement with that determined by WES.[15,16] MSI and TMB are predictive biomarkers of the efficacy of ICIs in clinical practice. Furthermore, NGS-based MSI assays do not necessarily require the matching of normal tissues.[17] The concordance percentage of MSI-H and high TMB varies across different types of cancer.[18] In the present study, all MSI-H cases exhibited high TMB, whereas 49 of 319 (15.4%) MSS cases had high TMB, which is in agreement with the results of the previous study.[19] However, some studies have reported a highly variable response to ICIs, such as patients with MSS, negative PD-L1 expression, or low TMB showing a good response, or vice versa.[20,21] This suggests that other indicators may affect the response to ICIs. It has been shown that many factors including multiple intrinsic and extrinsic factors have been shown to affect the response to ICIs therapy.[20,21] Other promising predictive mutated genes include PLOE, POLD1, PTEN, and SKT11.[20-24] Therefore, a combination of multiple biomarkers may increase the predictive accuracy of ICI efficacy. Given that there is no standard TMB calculation and distinct cut-off values for different types of cancer,[20,25] further large-scale studies are necessary to standardize the TMB calculation method to ensure accuracy, reproducibility, reliability, and clinical utility. In addition to PD-L1 expression, a larger NGS panel can cover the majority of biomarkers for response to ICIs, which in turn helps comprehensively evaluate its efficacy.

Conclusion

In conclusion, a large NGS panel can provide comprehensive molecular evidence for precise cancer treatment and help in achieving precise management. Furthermore, with the continuous expansion of treatment plans and applicable populations of targeted drugs and ICIs, the prognosis of cancer patients has been greatly improved. However, drug resistance has become increasingly prominent. By comprehensively detecting the variant information of cancer-related genes, especially pathway genes, a large NGS panel can explore the mechanism of drug resistance to help patients benefit from long-term treatment. With the passage of time and cost reduction, large NGS panels will show increasingly broad applications in precision cancer medicine.

Financial support and sponsorship

This study was supported by the Key Project Foundation of Taizhou School of Clinical Medicine, Nanjing Medical University (grant No. TZKY20240302), and Taizhou Society Development Project, Jiangsu, China (grant No. TS202001).

Conflicts of interest

There are no conflicts of interest.

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