REVIEW

EXPERIMENTAL ONCOLOGY

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APPLICATION OF NEXT-GENERATION SEQUENCING TO REALIZE PRINCIPLES OF PRECISION THERAPY IN MANAGEMENT OF CANCER PATIENTS

All cancers are diseases of the genome, since the cancer cell genome typically consists of 10,000s of passenger alterations, 5—10 biologically relevant alterations, and 1—2 "actionable" alterations. Therefore, somatic mutations in cancer cells can have diagnostic, prognostic, and predictive value. Traditional methods are widely used for testing, such as immunohistochemistry, Sanger sequencing, and allele-specific PCR. However, due to the low throughput, these methods are focused exclusively on testing the most common mutations in target genes. The modern next generation sequencing (NGS) is a technology that enables precision oncology in its current form. ESCAT and ESMO Guidelines defined NGS for routine use in patients with advanced cancers such as non-squamous non-small cell lung cancer, prostate cancer, ovarian cancer, and cholangiocarcinoma. The high sensitivity of the NGS method allows it to be used to search for specific mutations in circulating tumor DNA in blood plasma and other body fluids. NGS testing has evolved from hotspot panels, actionable gene panels, and disease-specific panels to more comprehensive panels. The exome and whole genome sequencing approaches are just beginning to emerge, that is why panel-based testing remains most optimal in oncology practice. NGS is also widely used to identify new and rare mutations in cancer genes and detect inherited cancer mutations.

Keywords: precision oncology, next-generation sequencing, somatic and inherited mutations, liquid biopsy.

Cancer is the cause of one in six deaths worldwide [1]. It encompasses over 100 different diseases with diverse risk factors and epidemiology, originating in most cell types and organs of the human body and characterized by the uncontrolled cell proliferation and ability to modify the local microenvironment to favor its own proliferation, invade beyond normal tissue barriers, and metastasize to distant organs.

All cancers are diseases of the genome, since they develop because of the accumulated changes in the DNA sequence of cells [2]. The process of cancer development is similar to the Darwinian evolution during the origin of species, since it is based on two components: the continuous acquisition of genetic changes in individual cells due to random mutations and natural selection, which fixes certain phenotypic characteristics in the subsequent generations. On the one hand, selection can eliminate cells with harmful mutations, on the other hand, cells can appear that carry a number of fairly advantageous mutations that allow them to reproduce

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autonomously, be insensitive to apoptosis stimuli, have superiority in the struggle for growth factors, and survive more effectively than their neighbors, as well as penetrate into nearby tissues and metastasize to distant organs [3].

All somatic mutations that arise in a cancer cell, regardless of their structural nature, can be classified according to their consequences for cancer development. "Driver mutations", positively selected early in the malignancy process, confer a growth advantage to cells. They arise in the so-called cancer genes and as such are implicated in oncogenesis. Other mutations, which occur randomly during tumor progression and do not provide a growth advantage, are the "passengers".

A distinct subtype of driver mutations is represented by those conferring resistance to cancer therapy. Therapy resistance mutations are found as cancer progresses after successful prior treatment and may arise *de novo*. It is possible that some of these mutations already exist before therapy as passengers in a small subclone of tumor cells. Due to the changes in the selective environment associated with therapy, a "passenger mutation" can be converted into a "driver mutation", and the small subclone expands and manifests as relapse/progression.

The tumor cell genome typically consists of 10,000s of passenger alterations, 5—10 biologically relevant alterations, and 1—2 "actionable" alterations. An actionable alteration is the term given to a driver mutation with significant diagnostic, prognostic, or therapeutic implications in subsets of cancer patients and for specific therapies. All classes may be clinically relevant because they inform the physician about the molecular changes underlying the patient's disease [3].

Therefore, somatic mutations in tumor cells can have diagnostic value and be prognostic and predictive markers of response to targeted therapy. The use of molecular genetic diagnostics in oncology is particularly relevant due to the possibility of choosing optimal treatment regimens for patients depending on the molecular profile of the tumor, using a personalized approach, as well as identifying hereditary predisposition to oncological diseases for their timely prevention.

Precision medicine is an emerging treatment approach in oncology that considers the individual molecular features of the tumor [4]. The concept of precision oncology is not so new; 40 years have passed since the development of hormonal therapy for breast cancer. As known, its effectiveness is determined based on the predictive value of the expression of specific hormonal receptors. One of the first pieces of evidence of the relevance of a molecular-targeted approach in cancer therapy is the development of targeted therapy drugs such as trastuzumab for breast cancer or imatinib mesylate for patients with chronic myeloid leukemia.

Over the past few decades, the tests for individual gene profiling (molecular alterations) of various tumors, including lung, stomach, colorectal, breast, ovarian, and melanoma cancers, have been introduced into clinical practice, allowing patients with specific genomic alterations to have greater access to more effective treatments. The first phase of the genomic profiling era was limited to identifying molecular alterations, each of which could be detected by a specific test aimed at determining sensitivity/resistance to a single drug and for a specific site in the cancer. Currently, traditional approaches, including Sanger sequencing, pyrosequencing, and allele-specific PCR, are widely used for the DNA nucleotide sequence analysis [5]. However, due to low throughput, these methods are focused exclusively on testing the most common mutations in target genes.

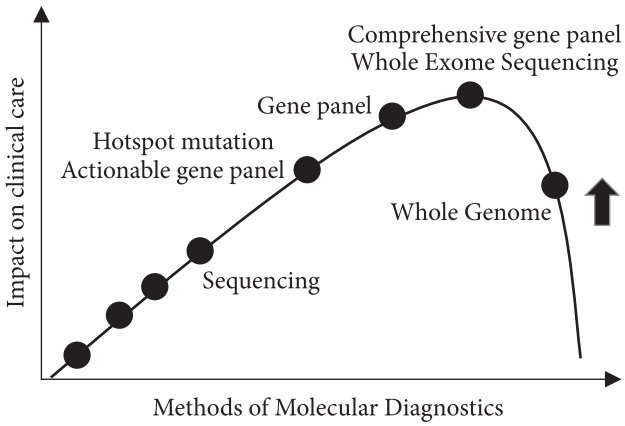
Tumor profiling uses a variety of techniques to analyze the tumor sample and return information about the genes altered in the cancer cells (Figure). Immunohistochemistry (IHC) determines the level of protein expression. Traditional IHC is based on the immunostaining of thin sections of paraffin-embedded tissues fixed on glass slides [6]. Typically, IHC slides are prepared, processed, and stained manually in small portions or using automation options for high-throughput sample preparation and staining. Light or fluorescence microscopy is used to study tumor samples, as well as digital approaches — image capture and subsequent interpretation by a pathologist or using specialized programs, the so-called digital pathology. IHC is an important additional tool for the differential diagnoses of tumors that are not determined by the conventional analysis of hematoxylin-eosin-stained samples as well as for scientific studies.

In situ hybridization (ISH) is a powerful cytogenetic method that detects gene deletions, amplifications, translocations, and fusions. Today there are two basic ways to visualize RNA and DNA targets *in situ*, namely, fluorescence (FISH) and chromo-

genic (CISH) detection. This method is characterized by rapid turnaround time and is more accurate than IHC and result interpretation is less subjective. FISH/CISH is performed on tumor sections. Fluorescent probes complementary to DNA (or RNA) are used to identify the desired targets. Hybridization of the probe with the target, which can be genes, chromosome sections, or whole chromosomes, is performed. It reveals deletions, gene amplifications, translocations, and fusions [7].

Polymerase chain reaction (PCR) is a method of detecting a specific region of nucleic acids in biological material by amplification (multiple increase in the number of copies of the studied fragment) in vitro [8]. This method is widely used by some guidelines for linking diseases to the FDA-approved targeted therapies as a satellite test. It tends to be of high specificity and sensitivity in amplification of the target sequence of DNA fragment, is cheap and readily accessible, and characterized by rapid turnaround time. Nevertheless, it detects only known clinically significant mutations, the number of which is constantly increasing. This requires continuous optimization of PCR test systems.

Sequencing is the process of determining the order of nucleotide bases (A, C, T, G) in a DNA sequence. Sanger sequencing examines strands of DNA to identify mutations by analyzing long contiguous sequencing reads [9]. In Sanger sequencing, the target DNA is copied many times making fragments of different lengths. Fluorescent "chain terminator" nucleotides mark the ends of the fragments and allow the sequence to be determined. The fragments produced are then separated by size using capillary electrophoresis. They enter the detector in order of size from shortest to longest. The laser and detector analyze the fluorescent ddNTP at the end of each strand, creating a color indication. Since the sequencing reaction produces chains of all possible sizes, it allows the original sequence to be identified. The advantage of the method is the ability to produce long reads of more than 500 bases. Nevertheless, there are some disadvantages, namely, throughput is poor compared to NGS: at about 100—200 kilobases per hour vs. several hundred megabases/gigabases per hour for leading NGS systems. The use of gels/polymers as separation media makes dealing with large sequencing outputs challenging and limits the number of samples that can be handled.



The evolution of molecular testing in practical oncology

More precise and extensive characterization of tumors using gene profiling allows for a better understanding of the molecular mechanisms underlying tumor growth, enabling the development of more personalized therapeutic options. Accordingly, there has been a marked increase in the number of targeted therapies approved for the treatment of patients with specific types of malignancies harboring specific types of sequence alterations. Therefore, with the development of precision oncology, there appeared a need to diagnose several molecular changes using different methods. For example, patients with advanced or metastatic colorectal cancer with wild-type KRAS are treated with monoclonal antibodies to the epidermal growth factor receptor (EGFR) such as cetuximab or panitumumab. These patients often develop resistance to these drugs when activating mutations arise in the *KRAS*, *NRAS*, *BRAF*, or *PIK3CA* genes [10].

A prime example of the use of a hotspot panel is non-small-cell lung cancer (NSCLC). Since the initial approval of targeted agents, such as erlotinib and gefitinib, for patients with activated *EGFR* mutations a decade ago and the recent approval of crizotinib for patients with *ALK* gene fusions, the routine genetic testing for these mutations in lung cancer biopsies or liquid biopsies samples has become a standard diagnostics option for providing the optimal patient care [11].

The College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) have issued joint evidence-based guidelines for molecular testing in lung cancer. These guidelines recommend testing all patients

with lung adenocarcinoma for *EGFR* and *ALK* abnormalities, regardless of clinical parameters such as smoking history, gender, or ethnicity, to determine whether EGFR inhibitor or ALK inhibitor therapy may be beneficial.

From the end of the 20th century for the following 25 years or so, Sanger sequencing was the most widely used technology for decoding of nucleic acid sequences. Currently, Sanger sequencing has been superseded by next-generation sequencing (NGS) technology [12].

NGS is a highly productive technology based on massive parallel sequencing of specially prepared single-stranded libraries of fragmented DNA of the samples under study. Several commercial platforms are currently available for high-throughput sequencing, namely HiSeq, MiSeq and NextSeq 500 (Illumina), Roche 454 GS, Ion Torrent (Thermo Fisher Scientific), and SOLiD (Applied Biosystems). Most high-throughput sequencing technologies include the following steps: library preparation, sequencing itself, and analysis of the obtained data. Library preparation involves fragmenting DNA to 300—500 base pairs, ligating sequencing adapters (synthesized oligonucleotides with a known sequence) to the ends of the fragments, and amplifying the resulting libraries. The amplification method may differ between platforms. For example, Thermo Fisher Scientific's Ion Torrent platform uses an emulsion PCR to amplify single-stranded fragments on beads, while Illumina technology amplifies fragments using a bridge PCR and forms clusters on a flow cell.

Sequencing is performed by synthesizing new DNA fragments on single-stranded DNA libraries, which act as a template. Nucleotides are incorporated into the new chain in a specific order corresponding to the template chain, which is recorded digitally. After each subsequent nucleotide is incorporated into the chain, the device records a signal. Different NGS platforms use different mechanisms for detecting nucleotides incorporated into the new chain: detection of a fluorescent signal after incorporation of a nucleotide complementary to the template into the chain (Illumina, SOLiD Applied Biosystems), a change in the pH of the solution in the microreactor associated with the release of hydrogen ions into the medium during DNA synthesis by the enzyme (Ion Torrent, Thermo Fisher Scientific), or recording a light signal after the release of pyrophosphate, which activates a cascade of chemical reactions (Roche).

After sequencing, the obtained data must be processed either using bioinformatics or special software installed on the device or server (Variant Studio, Illumina; Ion Reporter, Thermo Fisher Scientific). The data undergo several stages of processing, namely, exclusion of reads with low read quality, alignment of data relative to the reference sequence or *de novo* sequence assembly, and analysis of the sequencing results, which allows determining the type of genetic variants, including their hereditary nature, assessing the level of gene expression, and identifying new genes and regulatory elements.

New sequencing technologies have improved the Sanger electrophoresis-based method, which was discovered more than 30 years ago and whose disadvantages include low throughput and relatively high cost. Today, NGS is the dominant technology in genomic research and has been rapidly implemented in clinical oncology [13]. Due to its unique capabilities, NGS ideally meets the needs of clinical diagnostics and allows for large arrays of studies at lower costs. Therefore, NGS technology is becoming a driving force for the development of precision medicine in oncology. Due to the massively parallel principle, the sequencing time, labor costs, and reagents are reduced, which leads to significant savings. NGS has high sensitivity and allows detecting >1% of mutations, which is critical for the detection of somatic mutations in heterogeneous samples of solid tumors. Modern NGS technology uses just nanograms of tumor DNA to build libraries. Both MiSeq and Ion PGM can sequence about 50 target genes from 10—50 ng of DNA extracted from formalin-fixed paraffin-embedded (FFPE) samples. This is particularly important, since in many clinical situations, the only available specimen is often a fine needle aspiration biopsy or FFPE tissue slides, which do not provide sufficient DNA for classical Sanger sequencing. With NGS technology, multiple genes can be reliably sequenced with high coverage.

The official completion of the Cancer Genome Project in 2014 led many to envision the clinical implementation of cancer genomic data as the next logical step in cancer therapy. Stemming from this vision, the term "precision oncology" was coined to illustrate the novelty of this individualized approach. The basic assumption of precision oncolo-

gy is that molecular markers detected by NGS will predict a response to targeted therapies independently of the tumor histology. NGS has led to a better characterization of many cancers, leading to the identification of new subtypes, biomarkers search, and identification of new therapeutic targets, culminating in the completion of The Cancer Genome Atlas (TCGA; http://cancergenome.nih.gov) [13].

Additional projects dedicated to NGS cancer research have been initiated such as Therapeutically Applicable Research to Generate Effective Treatments (TARGET) for childhood cancers and the International Cancer Genomics Consortium (ICGC; https://dcc.icgc.org) for adult cancers. TCGA and ICGC projects are to generate comprehensive whole genome sequencing (WGS) data from more than 25,000 tumors. The Catalogue of Somatic Mutations in Cancer (COSMIC) database now contains somatic mutation information from over a million of tumor specimens and is continually updated. NGS data from these large projects have identified a number of recurrent genomic aberrations across multiple tumor types and have revealed a "long tail" of rare, but in many cases actionable, mutations. Currently, several strategies have been developed for NGS in cancer ranging from the targeted gene panels covering several thousand base calls through the whole exome sequencing (WES) of ~22,000 coding genes (40—50 million bases) to WGS (3.3 billion bases) of the human genome. NGS found its first clinical application in 2011, when the feasibility of integrating WGS, WES, and transcriptome sequencing into oncology decision-making was demonstrated. WGS, WES, and even targeted sequencing can be used to search for new genetic aberrations and associated potential therapeutic targets for various tumors.

NGS can be used for WGS, WES, transcriptome sequencing (mRNA sequencing), and targeted sequencing of multigene panels [13, 14]. According to the surveys conducted by AMP, the American College of Medical Genetics and Genomics, the American Society of Clinical Oncology, and CAP to determine the use of NGS in clinical practice, a minority of laboratories perform exome (12%) or genome (5%) analysis on tumor tissue. WGS produces a large amount of data on single-nucleotide variants, insertions and deletions (indels), structural arrangements, gene fusions, and copy number variations, which requires complex bioinformatics analyses and support. It is expensive to implement this technique as a standard clinical assay and, given its high sensitivity, incidentally discovered novel variants are frequently of uncertain clinical significance [15]. In contrast, targeted NGS is more affordable, efficient, and suitable for clinical use. The design of a targeted panel largely depends on the purpose of the panel — whether it is clinical testing for available approved therapeutic targets or testing in the context of clinical trials. Therefore, targeted NGS panels can range from hotspot panels of a limited number of codons to large panels that include the entire coding regions of hundreds of genes.

The hotspot panel is a set of frequently mutating hotspots that have either clinical significance or diagnostic value. A typical example is lung cancer. The majority of lung cancers are diagnosed at an advanced stage, when the opportunity to receive surgical resection is missed. Therefore, for the diagnosis and therapeutic decision making, either small biopsies or cytology samples are used. A wide range of targeted drugs has been developed for the treatment of lung cancer. Therefore, diagnostic testing should cover the following potential target genes: *EGFR, BRAF, KRAS, ALK,* and *ROS1*. More potential targets, such as *PIK3CA, FGFR1*, and *DDR2,* are under clinical investigation. Therefore, the number of predictive satellite biomarkers for novel targeted drugs is expected to continuously increase. Such analysis is difficult to achieve by Sanger sequencing, both practically (too long and costs too much) and technically (not enough tissue and low sensitivity). In addition to sequencing or PCR, it is also necessary to use FISH for translocations and fusions. Often, an insufficient amount of tumor material does not allow performing all the necessary diagnostic studies. Thus, the NGS technology is superior to current standard methodologies in lung cancer treatment [16, 17].

In June 2017, the FDA approved Oncomine Dx Target Test (Thermo Fisher Scientific), a 23-gene panel, as the first NGS-based companion diagnostic that can be used to select patients for three FDA-approved therapies for NSCLC: gefitinib (EGFR), crizotinib (ALK and ROS1), and the combination of dabrafenib and trametinib (BRAF) in NSCLC. The National Cancer Institute of Ukraine has im-

plemented diagnostics NGS panel, Oncomine Focus Assay (Thermo Fisher Scientific), which is a targeted, multi-biomarker assay that enables detection of targets-relevant hotspots, SNVs, indels, CNVs, and gene fusions from DNA and RNA in a single workflow. This panel enables simultaneous detection of hundreds of variants across 52 genes relevant to solid tumors including colorectal cancer and NSCLC.

Now, actionable gene panels have evolved from hotspot panels by including all exons of the target genes or all clinically relevant regions to allow examination of other pathogenic mutations beyond the frequently mutated regions. A common feature of these panels is a harnessing of actionable genes such as *KRAS, NRAS, BRAF, EGFR, PIK3CA, KIT, ALK,* and *ROS1*, which are frequently targeted by FDA-approved drugs in a variety of tumor types.

The entire coding region of the genome (exome) represents only about 1—2% of the genome but contains about 85% of the pathogenic variants that cause disease. The feasibility of WES for clinical use is currently under debate, and eventual acceptance of this approach appears imminent.

WGS produces a large amount of data on single-nucleotide variants, insertions and deletions (indels), structural arrangements, and copy number variations that require complex bioinformatics analysis and support. It is expensive to implement this technique as a standard clinical assay and, given its high sensitivity, the significance of incidentally discovered novel variants is frequently of uncertain clinical significance.

Hereditary cancer susceptibility panel testing is currently very popular. Approximately 5—10% of all cancers are hereditary. More than 100 cancer susceptibility syndromes have been reported, including hereditary breast and ovarian cancer syndrome (HBOC), Lynch syndrome, Cowden syndrome (CS), and Li-Fraumeni syndrome (LFS) [18, 19]. Many of risk genes play a role in DNA damage repair, such as high-risk genes *BRCA1* and *BRCA2* and moderate-risk genes *BRIP1* and *PALB2*, which are part of the Fanconi anemia (FA)-BRCA molecular pathway and are associated with an increased risk of breast and ovarian cancer. NGS-based screening for all these genes for a particular cancer type provides important risk information for preventive measures. These panels typically have a limited set of genes allowing for multiplexity and greater depth of coverage to improve analytical sensitivity and specificity and reduce cost.

Identification of germline mutations in the highly penetrant *BRCA1* and *BRCA2* genes, which play a key role in maintaining genome integrity, in particular, in DNA repair processes in breast cancer, as well as in other highly and moderately penetrant genes associated with this oncopathology, is of great importance for choosing individual treatment regimens for patients, including preventive surgeries and the use of cytostatics and PARP inhibitors.

Until a certain time, the Sanger genetic testing method, which was considered the "gold" standard for identifying genetic disorders, was widely used to analyze genes associated with hereditary oncological diseases. However, it is only suitable for searching for known, most common mutations and is not suitable for sequencing entire genes due to its low throughput and high cost of this study. NGS allows for the detection of rare genetic variants and the simultaneous testing of a large number of predisposition genes for clinically significant mutations in a short time, in contrast to the traditional molecular genetic methods [13, 15, 16].

Modern NGS is a technology that enables precision oncology in its current form. As genomic research has accelerated the pace of target discovery for disease treatment, the number of such targets that need to be tested is continually increasing. This has resulted in an increasing number of targeted drugs approved for the treatment of patients with certain types of cancer that harbor certain types of genetic alterations. The ESMO Scale for Clinical Actionability of Molecular Targets (ESCAT) collaborative project, initiated by the ESMO Translational Research and Precision Medicine Working Group, provides a systematic framework for the assessment of molecular alterations as clinical targets [20]. ESCAT defines evidence-based clinical criteria to define genomic alterations as markers for patient selection for targeted therapies.

Molecular tests underlie the prescription of EGFR, BRAF, ALK, ROS1, and PARP inhibitors, as well as the use of some other cytotoxic and targeted drugs. In this regard, NGS tests performed to identify biomarkers approved in clinical practice are part of the traditional diagnostic procedures. The use of NGS technology allows one to optimize the use of small tissue samples and/or to detect newly

characterized changes that cannot be detected by other methods. According to current recommendations, NGS methods should be applied in clinical practice in selected advanced neoplasms depending on the number of molecular targets to be detected and their complexity. Based on these evaluation elements and the levels of clinical evidence of molecular targets according to ESCAT and ESMO Guidelines for the implementation of NGS in patients with metastatic cancer, non-squamous non-small cell lung cancer, prostate cancer, ovarian cancer, and cholangiocarcinoma are identified for routine use of NGS in samples from patients with advanced neoplastic disease [16, 21, 22]. In addition, in such tumors, the use of NGS technology allows optimizing the use of small tissue samples and/or detecting newly characterized alterations that cannot be detected by other assays. For such tumors, large multigene panels can be used if they add an acceptable additional cost compared to small panels. In colorectal cancer, NGS may become an alternative to PCR.

In 2021, the ESMO-EURACAN-GENTURIS Clinical Practice Guideline for diagnosis, treatment, and follow-up of gastrointestinal stromal tumors and the ESMO Clinical Practice Guideline for the diagnosis, staging, and treatment of patients with metastatic breast cancer integrated ESCAT scores for genomic alterations with actionable drug matches [23, 24]. In 2022, the ESCAT scores were included in updated thyroid cancer, new gastric cancer, esophageal cancer, metastatic colorectal cancer, salivary gland cancer, and biliary tract cancer treatment recommendations. In 2023, the ESCAT scores were included in treatment recommendations for oncogene-addicted metastatic non-small cell lung cancer, epithelial ovarian cancer, pancreatic cancer, and early breast cancer. In addition, the working group recognizes the relevance of using NGS technologies for the differential diagnosis of sarcomas.

Furthermore, based on the KN158 study, the tumor mutational burden (TMB) testing is recommended in cervical cancer, neuroendocrine tumors, salivary gland cancer, thyroid cancer, and vulvar cancer, as TMB is highly predictive of response to anti-programmed cell death 1 (anti-PD1) antibodies in these cancers [25]. In addition, ESMO recommends that clinical trial centers develop multigene sequencing as a tool to screen patients eligible for clinical trials and to accelerate drug development.

The implementation of NGS in pediatric oncology has the potential to impact diagnosis, therapeutic strategies, prognosis, clinical trial recruitment, and germline risk [26, 27]. Several recently published studies have demonstrated that the comprehensive sequencing (whole genome, whole exome, and whole transcriptome) of childhood cancers can identify clinically relevant alterations in a significant cohort of children with cancer and that some patients with actionable tumor alterations will benefit from and respond to appropriate targeted therapy.

The multicenter prospective iCat2/Genomic Assessment Informs Novel Therapy Consortium (GAIN) Study performed targeted NGS from FFPE tumor samples and collected clinical and personal data to assess the impact of the findings on patient outcomes. In the cohort of 345 children, 298 patients (86%) had one or more alterations with potential to impact treatment [28]. Genomic alterations with diagnostic, prognostic, or therapeutic value were present in 61%, 16%, and 65% of patients, respectively. After results were returned, the impact on the treatment included 17 patients with a refined diagnostic classification and 240 patients with a tumor molecular profiling result that could be used to select molecularly targeted therapy corresponding to the identified alterations. Of 29 patients who received targeted therapy, 24% had an objective response or durable clinical benefit.

The National Cancer Institute of Ukraine, with the support of the Tabletochki Charitable Foundation, initiated an all-Ukrainian project to study tumor genetic profiles to individualize therapy for children with solid malignant tumors of high-risk groups. The purpose of the project was to determine the genetic profile of the tumor using NGS and establish the significance of the identified genetic alterations for clarifying the diagnosis, prognosis, and individualizing the treatment tactics of this group of patients. Using NGS, the comprehensive genetic profiling of tumor cells with analysis of 200+ genes is performed, in particular, whole-exome and hotspots sequencing of tumor suppressor genes and oncogenes that frequently mutate, as well as transcriptome sequencing of target genes, and analysis of tandem repeat blocks involved in the formation of microsatellite instability are performed. "The Oncomine Childhood Cancer Re-

search Assay" and "TrueMark MSI Assay" (Thermo Fisher Scientific) are used for molecular profiling. Based on a comprehensive analysis of NGS tumor data, individualized medical recommendations for the appointment of targeted and/or immunotherapy, clarification of the diagnosis, and prognosis of the disease will be substantiated. In addition, differential diagnosis of soft tissue and bone sarcomas and tumors of the central nervous system in children will be improved. The biological component of the risk groups stratification system for pediatric patients with solid tumors will be supplemented. Preliminary results involving FFPE of 20 patients with high-risk solid cancers have made it possible to clarify the medulloblastoma subtype in one patient, to confirm the diagnosis of alveolar rhabdomyosarcoma in one patient, and to identify genetic alterations in two patients, which is an indication for inclusion of patients in clinical trials.

The high sensitivity of the NGS method allows it to be used to search for specific mutations in circulating tumor DNA in blood plasma and other liquids of the body [29, 30]. Tumors almost always release their fragments (individual cells or clusters of cells, DNA, RNA, proteins) into various body fluids. The so-called liquid biopsy, i.e., the analysis of circulating DNA (ctDNA) or some other molecules obtained from the tumor, holds great promise for non-invasive monitoring of cancer, analysis of mutations that cause drug sensitivity, and early detection of cancer. The analysis of ctDNA has been shown to diagnose minimal residual disease weeks earlier than radiologic investigation, and ctDNA-positive patients had a higher risk of recurrence, shorter overall survival, and disease-free survival compared to ctDNA-negative patients. The utility of liquid biopsy and NGS for detection of anti-EGFR therapy resistance mutations in advanced/metastatic lung and colorectal cancer has been recently described. Liquid biopsy possesses a strong advantage over tissue biopsy because it provides the real-time genomic status of the tumor and possibilities for frequent sampling due to minimal invasiveness.

NGS is widely used to identify new and rare mutations in cancer genes, detect inherited cancer mutations, and is also widely used in practice to personalize cancer treatment by providing biomarkers for appropriate targeted therapy [31, 32]. Combining NGS and liquid biopsy techniques enables obtaining a comprehensive genomic profile of a tumor in a single assay using the same biological sample. This new approach leads to the selection of increasingly precise candidates for targeted therapy and then to the monitoring of their treatment along with the identification of resistant tumor clones.

NGS testing has evolved from hotspot panels, actionable gene panels, and disease-specific panels to more comprehensive panels. Exome and whole genome sequencing approaches are just emerging. Due to the incompleteness of clinical implementation of human genome sequencing data, a panelbased testing is currently more optimal and occupies a well-established place in oncology practice.

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ЗАСТОСУВАННЯ МЕТОДУ СЕКВЕНУВАННЯ НОВОГО ПОКОЛІННЯ ДЛЯ РЕАЛІЗАЦІЇ ПРИНЦИПІВ ПРЕЦИЗІЙНОЇ ТЕРАПІЇ В НАДАННІ МЕДИЧНОЇ ДОПОМОГИ ОНКОЛОГІЧНИМ ХВОРИМ

Усі види раку є захворюваннями геному, оскільки геном пухлинної клітини зазвичай складається з десятків тисяч мутацій-пасажирів, 5—10 біологічно значущих мутацій та 1—2 «активуючих» мутацій. Тому соматичні мутації в пухлинних клітинах можуть мати діагностичне, прогностичне та предиктивне значення. Традиційні методи широко використовуються для досліджень, такі як імуногістохімія, секвенування за Сенгером та алель-специфічна ПЛР. Однак через низьку пропускну здатність ці методи зосереджені виключно на тестуванні найпоширеніших мутацій у цільових генах. Сучасна NGS — це технологія, яка дає змогу дотримуватися принципів прецизійної онкології в її нинішньому вигляді. Рекомендації ESCAT і ESMO визначили NGS для рутинного застосування у пацієнтів із розповсюдженими формами раку, такими як неплоскоклітинний недрібноклітинний рак легені, рак простати, рак яєчників і холангіокарцинома. Висока чутливість методу NGS дозволяє використовувати його для пошуку специфічних мутацій в циркулюючій пухлинній ДНК у плазмі крові та інших рідинах організму. Тестування NGS еволюціонувало від застосування панелей гарячих точок, панелей активуючих мутацій в генах і панелей для конкретних захворювань до більш комплексних панелей. Підходи до секвенування екзомів і цілого геному тільки починають з'являтися, тому панельне тестування залишається найбільш оптимальним в онкологічній практиці. NGS також широко використовується для виявлення нових і рідкісних мутацій у генах, асоційованих із розвитком раку, а також спадкових мутацій, що визначають схильність до розвитку онкологічних захворювань.

Ключові слова: прецизійна онкологія, секвенування нового покоління, соматичні та спадкові мутації, рідинна біопсія.