Next-Generation Sequencing for the General Cancer Patient

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Abstract: Next-generation sequencing is a novel method of DNA sequencing that has become a cornerstone of precision oncology. This sequencing method detects differences in specific DNA sequences between a sample and a reference genome or matched normal DNA. In addition to single-nucleotide variants, other insertions, deletions, copy number changes, and fusions may be drivers of cancer growth, and thus represent therapeutic opportunities. As a result, genomic characterization has been increasingly used to guide treatment decisions, especially in patients with advanced disease. This review discusses the basic technologies involved in next-generation sequencing, the applications of this method, and limitations in the clinical realm.

Introduction

Genomic sequencing has become increasingly available over the past few decades. In 1976, a bacterial genome was sequenced for the first time via a method known as Sanger sequencing. Since then, a faster DNA sequencing method known as next-generation sequencing (NGS) has emerged.[1] The clinical application of NGS detects differences in a patient's genome from a reference or normal genome. It identifies sections of DNA that represent changes (variants), including insertions or deletions in a specific DNA sequence or array of sequences.[1] These areas of identifiable change have become areas of potential study, clinical diagnostic tools, and therapeutic targets.

The genome and transcriptome of tumors or individual cells can be profiled using the sequencing technologies detailed below, allowing for the study of specific oncogenic pathways. The process has continued to evolve; a recent advance is the simultaneous evaluation of DNA and RNA from the same cell. A multiomics approach will allow the real-time generation of mechanistic models to pin genomic variation against protein expression, which in turn should allow a more comprehensive understanding of cellular behavior in various tumor types.

In modern oncology, sequencing for tumor-specific genomic alterations via panel testing has become more widespread. Many oncologists have begun to use commercial tests, such as FoundationOne CDx (Foundation Medicine). Some institutions have implemented commercial platforms such as the Oncomine Comprehensive Assay

(ThermoFisher) and a small subset of institutions have developed their own platforms, such as the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay.

This review will discuss the basic technologies in the application of NGS and assess the importance of various genetic alterations and their applicability in the clinical realm. It will further discuss current limitations and future directions as sequencing technology evolves.

How Next-Generation Sequencing Works

The sequencing process uses various platforms. Below is a discussion of the most commonly used methods across different platforms available today: DNA sequencing, RNA sequencing, single-cell RNA and DNA sequencing, and liquid biopsy.

DNA Sequencing

DNA sequencing includes sequencing by synthesis, pyrosequencing, and sequencing by ligation.

Sequencing by Synthesis. Sequencing by synthesis involves the incorporation of reversibly fluorescent and terminated nucleotides in DNA sequencing. Here, each nucleotide is reversibly attached to a single fluorescent molecule with unique emission wavelengths. Eventually the nucleotide is reversibly terminated, ensuring a single nucleotide per cycle event. First, small genomic sections are flagged by man-made target sequences.[2] These sequences bind the patient's DNA at fixed points on a glass slide to prevent mobility. From here, single bases with a fluorescent tag are added to DNA mix. These bases find the target sequence and initiate a complementary strand to the patient DNA, creating long sequences of double-stranded DNA.[2] A fluoroscopy microscope captures light off each individual fluorescent tag, mapping the genome base by base.[2] At the end of sections called terminator groups, sections are cleaved and washed away. This process is repeated over multiple cycles until the sequencing reaction is complete. The advantage here lies in the incorporation of a single nucleotide at a time, and the ability to clear sequences separately before evaluation of the subsequent sequence.

Pyrosequencing. In pyrosequencing, the sequencing reaction is begun with target sequences—just like in sequencing by synthesis. Afterward, individual nucleotides are added that are bound to pyrophosphate.[3] As nucleotides are added one at a time, their incorporation into the DNA template results in the release of pyrophosphate, resulting in the generation of light. The emission of this light is detected by a camera, which records the appropriate sequence of the cluster. Any unincorporated bases are degraded by an enzyme called apyrase before any subsequent nucleotides are added.[3] This cycle continues until the sequencing reaction is complete.

Sequencing by Ligation. Sequencing by ligation varies from the other 2 methods because it does not utilize a DNA polymerase to incorporate nucleotides. Rather, short sections of nucleotides called oligonucleotides are bound to one another. These oligonucleotides are made of 8 bases, 2 of which are used as starter probes. The remaining bases are attached to the subsequent probes.[3] The end of each of these probes is attached to 1 of 4 fluorescent dyes. The reaction begins by binding the primer to the adapter sequence. Subsequent primer probes attach to the adapter sequence and are ligated to the primer sequence through an enzyme called DNA ligase. Those oligonucleotides that are not used are removed. The signal of fluorescent dye on the last 3 nucleotides is detected and recorded. Then the fluorescent signal is cleaved and the next cycle commences. After several cycles of ligation, the DNA strand is denatured. Finally, the process begins anew with another primer that is off by one base from the prior primer, and the steps are repeated.

The output of each of these sequencing platforms results in several reads from each of the long strands created. Each machine provides these raw data at the end of a sequencing run. These data are then further analyzed to draw meaningful conclusions.

RNA Sequencing

The introduction of high-throughput NGS technology has also revolutionized transcriptome medicine. Here, RNA is extracted from the biological material of choice, such as cells or tissue. Second, subsets of clinically active RNA molecules are isolated using a variety of protocols. These include poly-A selection to siphon polyadenylated transcripts, and ribo-depletion to remove ribosomal RNAs. Following one of these protocols, RNA is converted into complementary DNA via reverse transcription. Finally, these chains are amplified by polymerase chain reaction with fluorescent complements. The data are then ready for sequencing in a similar fashion to DNA through addition of individual nucleotides and read out with use of fluoroscopy as above.[4] RNA sequencing has the advantage of being able to provide information on RNA expression, as well as to detect fusions and alternative splicing. Although RNA sequencing can also theoretically detect mutations, DNA is often preferred for mutation detection.

Single-Cell RNA and DNA Sequencing

Sequencing of one molecular subtype—either RNA or DNA—from a single cell can give us cell-specific insight. The first key step is to generate a single-cell suspension

Table 1. Comparison of Sequencing Platforms

Platform (Manufacturer)	No. of Genes Assessed	Tissue Required	Mutations	Analysis Type (Tumor, Tumor vs Normal)
FoundationOne CDx (Foundation Medicine)	324	10 slides and >20% tumor	• DNA sequencing: copy number alterations, gene fusions, MSI, TMB	Tumor
Caris CDx (Caris Life Sciences)	592	10 slides and >20% tumor	• DNA sequencing: copy number alterations, MSI, TMB • RNA sequencing: gene fusions, mRNA variants	Tumor
TempusXT (Tempus)	596	10 slides	• DNA sequencing: copy number alterations, gene fusions, MSI, TMB	Tumor vs normal
Oncomine Comprehensive Assay (ThermoFisher)	161	1 H&E, 20 unstained slides and >20% tumor	• DNA sequencing: copy number alterations, gene fusions	Tumor or tumor vs normal
PCDx (Paradigm)	500	1 H&E, 1 IHC and 2 unstained slides with >15% tumor	• DNA sequencing: copy number alterations, gene fusions, MSI, TMB	Tumor
GPS Cancer (NantOmics)	20000 genes + RNA	10% unstained and >25% tumor	• RNA sequencing: copy number alterations, gene fusions, MSI, TMB	Tumor vs normal
CancerPlex (Kew)	>400	1 H&E and 10 unstained slides	• DNA sequencing: copy number alterations, gene fusions, MSI, TMB	Tumor
Guardant360 (Guardant)	76	NA	• DNA sequencing: copy number alterations, 6 gene fusions	Plasma
FoundationOne Liquid (Foundation Medicine)	70	NA	• DNA sequencing: copy number alterations, specific gene fusions for lung malignancies, MSI	Plasma

H&E, hematoxylin & eosin; IHC, immunohistochemistry; MSI, microsatellite instability; NA, not applicable; TMB, tumor mutation burden.

for analysis. This can be done via manual isolation using micromanipulation equipment to obtain a single cell or via a subset of cells to undergo one division, giving rise to monoclonal daughter cells. Then, using fluorescence-activated cell sorting, phenotypically distinct cells or even nuclei can be sorted apart from their cytoplasm to obtain either DNA or RNA.[5] New microfluidic technology also has been used to isolate messenger RNA (mRNA) from nuclei into individual capture sites and initiate amplification. New microfluidic approaches allow single cells or nuclei to be encapsulated within individual droplets prior to sequencing, called Drop-seq.[5] Following this, either RNA or DNA can be sequenced individually. Newer experimental approaches have been devised by which both RNA and DNA can be sequenced from the same cell simultaneously. These include gDNA and mRNA sequencing (DR-Seq) and genome and transcriptome sequencing (G&T-Seq). In DR-Seq, mRNA is transcribed to complementary DNA and amplified along with the nucleic DNA in the same vessel.[5] In G&T-Seq, the mRNA is separated via magnetic beading, and then transcribed and amplified separate from nucleic DNA.[5] Single-cell sequencing has unique advantages, including assessment of tumor heterogeneity and assessment of separate cell types, such as immune cells.

Liquid Biopsy

Interest is growing in obtaining DNA in a minimally invasive fashion from blood samples, either from plasma (also known as cell-free DNA) or from circulating tumor cells. This testing has been coined a "liquid biopsy." It was initially undertaken using allele-specific polymerase chain reaction and flow cytometry.[2] Now, NGS panels are increasingly being used, often with a focus on known mutations.[2]

Characteristics of Commercial Platforms

The characteristics of some of the more commonly utilized platforms are displayed in Table 1. These platforms

Table 2. Determinants of Actionability of a Genomic Alteration

Somatic or Germline Alteration
• Impacts the function of a cancer-related gene (activating oncogenes and inactivating tumor suppressor genes) and can be targeted directly or indirectly with approved or investigational therapies
• Meets specific eligibility criteria for enrollment into genotype-selected trials
• Has demonstrated the ability to establish diagnosis or influence prognosis
Germline Alteration
• Predicts drug metabolism and/or adverse effects
• Predicts future risk of cancer or other diseases (usually considered more "actionable" if prevention or screening with early treatment is feasible)

Adapted from Meric-Bernstam F et al. *J Natl Cancer Inst*. 2015;107(7): djv098.[14]

vary in number of genes assessed and types of mutations detected, as well as the amount and type of tissue utilized for analysis. Most platforms, including FoundationOne CDx (Foundation Medicine) and Caris CDx (Caris Life Sciences), analyze only the tumor tissue and include copy number variants, gene fusions, microsatellite genes, and tumor mutation burden as part of their analysis. Other platforms, such as TempusXT (Tempus), have developed tumor-vs-normal sequencing assays in which both tumor and nontumor genetics are compared for differences. Yet other platforms, such as Guardant360 (Guardant), have implemented liquid biopsy by analyzing the genetics of tumor cells in circulating plasma. As the genetics of individual solid tumors becomes better understood, the clinical utility of individual platforms may be found to differ between tumor types. At this time, however, these platforms cover most actionable alterations, and are designed to meet clinical needs in a tumor-agnostic fashion.

Clinical Applications: When to Order NGS

NGS has become very useful in clinical practice to detect actionable genomic alterations for both diagnostic and therapeutic purposes. As a general rule, NGS testing (like most medical testing) should be ordered when results may impact clinical management. For most disease types, the utility has predominantly been studied in patients with advanced disease—metastatic, locally advanced, or locally recurrent. For some mutations, such as *EGFR* mutations in lung cancer, *ALK* and *ROS* fusions in lung cancer, and *BRAF* V600 mutations in melanoma, a link exists to agents approved by the US Food and Drug Administration (FDA). In patients without biomarkers linked to an agent with an FDA indication, interest is growing in identifying genomic alterations that may represent investigational targets that could be leveraged for enrollment into genomically matched trials. Further, *TRK* fusions and microsatellite instability have both been approved as histology-agnostic biomarkers for FDA approval of larotrectinib (Vitrakvi, Loxo) and pembrolizumab (Keytruda, Merck), respectively.[6,7] These markers are detected in some but not all NGS platforms, emphasizing the need to know the differences between platforms and to keep in mind what is likely to be detected in different tumor types when initiating NGS testing.

Clinical Application: Interpreting Results

Determining Actionability

In the context of the management of a cancer patient, actionability refers to clinical utility—the answer to the question, Does this alteration impact my clinical management? Actionability could impact therapy selection by offering insights regarding therapeutic sensitivity or resistance, or by affecting diagnosis or prognosis (Table 2). In the context of precision oncology, the focus is predominantly on selection of therapy—either standard of care or investigational. Genomic biomarkers as a predictor of response/resistance will be the focus of the discussion below.

Functional Impact of Genomic Alterations

Single-Nucleotide Variants. Alterations in DNA sequence may affect the activity, localization, or expression of a gene, potentially affecting its function. Not all genomic alterations have the same functional impact. In general, alterations that increase the activity or expression of oncogenes and decrease the activity of tumor suppression genes are actionable. Thus, determining whether an alteration is expected to affect the function of a gene is crucial. This is especially important for missense mutations, as we increasingly have started using sequencing platforms that not only sequence the recurrent "hot spots" but also sequence the entire gene (or at least the coding sequence). This leads to an increase in recognition of variants of unknown significance.

Missense alterations in DNA sequence can be synonymous (meaning that a different nucleotide sequence leads to no change in amino acid sequence) or nonsynonymous (meaning that a different nucleotide sequence leads to a change in amino acid sequence). Functionally activating mutations in oncogenes are often recurrent (such as *AKT1* E17K or *PIK3CA* H1047R). If the literature does not support an effect on clinical outcomes, or

preclinical data do not establish that an alteration affects function, then single-nucleotide variants (SNVs) are classified as variants of unknown significance. Determining whether the SNV is a recurrent alteration, in a critical domain, or at a site where other actionable alterations have been observed may also give some insight into the implications of an SNV and help increase the potential of actionability.[8]

Inactivating mutations in tumor suppressor genes can be more variable. Nonsense mutations leading to early truncation of a protein encoded by a tumor suppressor gene are likely to be inactivating, especially if truncation precedes or impacts functionally important domains.

Single nucleotide variants are also emerging as a common mechanism of resistance to small-molecule inhibitors. Mutations in *EGFR* and *ESR1* have been well described as mechanisms of resistance to EGFR inhibitors and endocrine therapy, respectively.[9,10] More recently, acquired mutations in *HER2* and *FGFR* were described in response to small-molecule inhibitors to human epidermal growth factor receptor 2 (HER2) and fibroblast growth factor receptor.[11,12]

Copy Number Variants. Larger deletions or amplifications in the genome are known as copy number variants or changes. Amplification of *ERBB2* gene encoding for HER2 is an example of a well-established oncogene, predominantly dysregulated through amplification and targeted through a variety of approved and investigational therapeutics.[13]

Copy number variants, however, can be more difficult to interpret given that large regions containing multiple genes can be amplified or deleted. The biological effect of the copy number gain or loss can be assessed preclinically with overexpression or small interfering RNA/short hairpin RNA showing knockdown of specific genes that impact function.[14] The clinical relevance of copy number changes is still being studied in many genes, however, with an emphasis on identifying thresholds for delineating clinically relevant amplifications. It is important to note that amplification on NGS often represents higher copy number alterations (often with 6 or 7 copies as reporting thresholds). Therefore, lack of amplification on NGS does not exclude the possibility of lower-level copy gain. The limitations in NGS reporting are also relevant when interpreting the presence or absence of deletions. Deletions in tumor suppressor genes such as *PTEN* are also likely to be important in cancer biology and therapeutic sensitivity. However, it is important to note that many NGS platforms have difficulty in reporting deletions, at least in part because of challenges in detection of deletions in tumors with low cellularity.

Gene Fusions. Gene fusions are a common cancer feature and result from the fusion of 2 or more genes, leading to translation of multiple proteins that have actionable changes in a cell.

Fusions increase the activity of genes known to be involved in tumor promotion. In oncology, this usually involves the fusion of a proto-oncogene, such as an activating kinase leading to increased protein function. Fusions have been found mostly in hematologic malignancies, as well as in bone and soft tissue sarcomas.[15] They have been less frequently found in epithelial carcinomas. However, some rare tumors have been associated with certain fusions. For example, *FGFR* fusion has been found in cholangiocarcinoma,[10] and *TRK* fusion has been found in a rare breast cancer subtype known as secretory breast cancer.[16]

Managing Multiple Actionable Alterations. If more than one copy number alteration is found and both (or more) are actionable, the target with strongest evidence for actionability (therapeutic sensitization) should be pursued. In addition, the mutation with the highest allelic frequency—which is expressed as a percent frequency—is usually the one pursued.[14]

The Interplay Between the Genome and the Transcriptome

The use of NGS for transcriptomics still is in its infancy. Eventually, the ability to survey both the genome and the transcriptome of individual cells in parallel will offer both clinical and experimental opportunities. First, it will directly link a germline or modified genotype of a cell to its phenotype, giving us insight into functional status. The causal effect of phenotypic expression will be directly linked to specific populations of cells without the potential confounding of vast numbers of cellular subpopulations. Further, longitudinal sequencing can be used to reconstruct a cellular lineage, which in oncology can help us predict how DNA will change over time to provide RNA sequences that occur during disease progression.

Incorporation of transcriptomics into precision medicine and integrated analysis with DNA-based NGS will likely refine precision oncology by confirming expression of actionable targets detected by genomics, and expanding actionability by identifying fusions and alterations of actionable genes at the transcriptional level.

Detection of Somatic vs Germline Mutations

Although pathogenic germline mutations (like those found in hereditary cancer syndromes, such as germline *BRCA* mutations) are present in all cells of the body,

mutations that occur only in the tumor are referred to as somatic. Optimally, to determine if a mutation is somatic or germline, patients would have NGS of blood or saliva from tumor and normal tissue. However, panels that combine tumor-only sequencing with a bioinformatics approach are being used to determine which alterations are somatic. For the clinical oncologist, it is critical to know what approach is used for reporting the NGS panel being reviewed, because a patient may have germline alterations that are not reported in the tumor-only sequencing. Some alterations on tumor-only panels that have been linked to hereditary cancers, such as mutations in *BRCA1* and *BRCA2,* may need to be investigated further with genetic counseling or testing.

Limitations

Limitations in NGS can occur with rare mutations, solid tumor biopsy, and liquid biopsy.

Rare Mutations

Even with the advances of NGS, inherent sequencing errors exist that cannot be avoided. New NGS approaches have improved sensitivity compared with Sanger sequencing, with many new NGS platforms reporting mutations found at 1% to 5% mutant allele frequency.[17] The sensitivity of testing is also impacted by tumor cellularity. Increasing the sensitivity of these technologies and implementing newer, noninvasive specimen collection techniques will give further insights into tumor heterogeneity and mechanisms of intrinsic and acquired resistance.

Solid Tumor Biopsy

Fresh frozen solid tumor tissue provides adequate quality samples for sequencing of the cancer genome. However, tissue samples are routinely formalin-fixed and paraffin-embedded for pathological examination and storage.[15] This can lead to significant degradation and changes to DNA and RNA. Formalin has been shown to add hydroxymethyl groups to nucleic acid bases and force cross-linking within proteins.[15] This can lead to alterations and errors in the output of the DNA being sequenced. However, with evolving technology, most DNA-based NGS platforms can be efficiently used on formalin-fixed paraffin-embedded samples.

Liquid Biopsy

Heterogeneity among tumors in the same individual (intertumor heterogeneity) as well as within a single tumor (intratumor heterogeneity) has been acknowledged as another challenge in precision medicine. Not only can metastatic tumors differ from the primary tumor, but biopsies from different sites can differ from each other. A strength of liquid biopsy is that it reflects the vast pool of alterations in a patient, assuming that the sample confers all available DNA.[2]

Liquid biopsies also have several limitations, however. The DNA sample is small and may be prone to missing small amounts of mutant DNA, such as in alterations of lower mutant allele frequency (subclonal), patients with limited disease burden, and tumor lineages that release only small amounts of DNA into circulation.[2] Further, certain alteration types, such as copy number changes, may be more difficult to identify.

As noted by several studies, liquid biopsy assays can vary in their identification of mutations. These differences may be attributable to under- or over-representation of certain mutations within a random liquid sample or to variations among the assays.

When comparing liquid biopsy with NGS sequencing from formalin-fixed paraffin-embedded samples, the detection rates of genomic alterations are often higher for tissue biopsies, with a small subset of cancers reporting comparable rates of detection. Some studies in NSCLC (detecting *EGFR*) and pancreatic cancers (detecting mutations such as *KRAS*, *TP53*, *APC*, and *SMAD4*) have reported sensitivity rates of 80% to 90% when comparing both forms of testing.[19,20] More recent prospective studies using matched tissue sampling reported a 46% rate of insufficient DNA sampling with liquid biopsy but a concordance rate of 79% for therapeutically targetable *EGFR* mutations in NSCLC,[22] and new orthogonal assays continue to improve mutation detection rates.[23] Further, liquid biopsies have the advantages of being noninvasive—thus facilitating serial sampling and providing insight into tumor heterogeneity.

Epigenetics

Although precision oncology has predominantly focused on genomics, gene expression can be modulated through a variety of mechanisms. Epigenetic modification of DNA is also involved in cancer causation. DNA methylation is the hallmark of epigenetic modification, and NGS can be applied to help detect how genes correlate to this methylation.[24] It is expected that this methylation status will provide information on future diagnosis and prognosis, and will predict therapeutic response. An example of DNA methylation profiling in action has been shown in NSCLC, where it found that hypermethylated regions are strongly associated with genes encoding transcriptional regulators.[25]

Clinical Counseling: Discussing Goals and Limitations

It is important for the oncologist to discuss the goals of NGS testing before initiating the test. Currently, 3 potential patient scenarios exist that can involve NGS use. The

first scenario is diagnostic, given that more tumors are now being defined and categorized by genetic mutations. The second application is for personalized medicine, in which the clinician can offer therapeutic options based on the presence or absence of a mutation. The third scenario is for patients who have an acquired resistance and are no longer responding to current standard therapy, in which case the clinician can offer experimental therapies based on availability.

A lack of empirical evidence exists regarding patient and clinician understanding of test results for either diagnostic or therapeutic purposes. Kaufman initially posed likely scenarios for patient delivery of NGS results, whereas Facio was among the first to survey adults regarding their expectations.[26,27] Further study is needed as to the best way to disclose NGS results to clinicians and providers. An important challenge is in the interpretation of NGS results, and thus decision support tools are critical in translating NGS testing into appropriate clinical action.[28,29]

It is important to set realistic expectations as well. Many patients who undergo testing do not have actionable alterations, and patients with actionable alterations may not be eligible for available therapies or ongoing trials—or if they are, the therapy may be ineffective or may not provide durable disease control. Finally, NGS has technical limitations in sequencing as well as in variant calling. The clinician should engage in an open dialogue with the patient on current limitations of using NGS.

It is also important for the oncologist to address the possibility of discovery of pathogenic germline alterations upon NGS testing.[30,31] The current guidelines from the American College of Medical Genetics and Genomics recommend that before sequencing, clinicians should inform patients of actionable variants amenable to personalized therapy.[32]

Clinicians using NGS are expected to discuss not only somatic findings but also findings that may have hereditary implications. Tumor testing on tumor-only platforms will not be able to determine whether an alteration is somatic or germline, so germline counseling should be considered. Notably, identification of mutations in *BRCA1* or *BRCA2* on tumor-only testing should trigger germline *BRCA1/2* testing even in the absence of a family history. Tumor testing is not optimized for germline testing, however, and is not a substitute for formal genetic counseling and testing when appropriate.

Personalized Medicine: Resources and Investigational Trials

The rapid progression of molecular profiling has made it difficult for oncologists to formulate real-time clinical decisions based on expansive evidence. To date, various parallel efforts have been created to generate knowledge bases to assist in interpretation of genomic testing results. These include national initiatives such as the Personalized Cancer Therapy website from the MD Anderson Cancer Center (www.personalizedcancertherapy.org), the Precision Medicine Knowledgebase from Weill Cornell Medicine (https://pmkb.weill.cornell.edu), and OncoKB from Memorial Sloan Kettering Cancer Center (http://oncokb.org/). All of these were created to provide an online resource for clinicians and researchers to facilitate navigation of available data.[2,33,34] These resources can be used to identify the therapeutic implications of specific oncogenic genomic alterations.

Conclusion

NGS has evolved over time and been integrated into clinical medicine, providing speed and accuracy for assessment of cancers with actionable genomic alterations. Its role in individualized medicine continues to be explored.

As NGS platforms become more readily available and affordable, biomarker-selected trials will become a priority to expedite advances in personalized medicine. Approaches such as liquid biopsies can be utilized to identify mechanisms of intrinsic and acquired resistance, and to design combinatorial therapies or novel therapeutics that overcome resistance. Over the next decade, transcriptomics and immune profiling are more likely to be incorporated into personalized profiling to better refine therapeutic options.

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References

1. Muzzey D, Evans EA, Lieber C. Understanding the basics of NGS: from mechanism to variant calling. *Curr Genet Med Rep*. 2015;3(4):158-165.
2. Kurnit KC, Bailey AM, Zeng J, et al. "Personalized cancer therapy": a publicly available precision oncology resource. *Cancer Res*. 2017;77(21):e123-e126.
3. Next generation sequencing (NGS): an introduction. ABM. https://www.abmgood.com/marketing/knowledge_base/next_generation_sequencing_introduction.php. Accessed July 1, 2019.
4. Kukurba KR, Montgomery SB. RNA sequencing and analysis. *Cold Spring Harb Protoc*. 2015;2015(11):951-969.
5. Macaulay IC, Ponting CP, Voet T. Single-cell multiomics: multiple measurements from single cells. *Trends Genet*. 2017;33(2):155-168.
6. FDA approves larotrectinib for solid tumors with NTRK gene fusions [press release]. US Food and Drug Administration. https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm626720.htm. Updated December 14, 2018. Accessed July 1, 2019.

7. FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication [press release]. US Food and Drug Administration. https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm560040.htm. Updated May 23, 2017. Accessed July 1, 2019.
8. Johnson A, Zeng J, Bailey AM, et al. The right drugs at the right time for the right patient: the MD Anderson precision oncology decision support platform. *Drug Discov Today*. 2015;20(12):1433-1438.
9. Yu HA, Arcila ME, Rekhtman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res*. 2013;19(8):2240-2247.
10. Javle M, Lowery M, Shroff RT, et al. Phase II study of BGJ398 in patients with FGFR-altered advanced cholangiocarcinoma. *J Clin Oncol*. 2018;36(3):276-282.
11. Rugo HS, Vidula N, Ma C. Improving response to hormone therapy in breast cancer: new targets, new therapeutic options. *Am Soc Clin Oncol Educ Book*. 2016;35:e40-e54.
12. Goyal L, Saha SK, Liu LY, et al. Polyclonal secondary FGFR2 mutations drive acquired resistance to FGFR inhibition in patients with FGFR2 fusion-positive cholangiocarcinoma. *Cancer Discov*. 2017;7(3):252-263.
13. Meric-Bernstam F, Johnson AM, Dumbrava EEI, et al. Advances in HER2-targeted therapy: novel agents and opportunities beyond breast and gastric cancer. *Clin Cancer Res*. 2019;25(7):2033-2041.
14. Meric-Bernstam F, Johnson A, Holla V, et al. A decision support framework for genomically informed investigational cancer therapy. *J Natl Cancer Inst*. 2015;107(7):djv098.
15. Xuan J, Yu Y, Qing T, Guo L, Shi L. Next-generation sequencing in the clinic: promises and challenges. *Cancer Lett*. 2013;340(2):284-295.
16. Euhus DM, Timmons CF, Tomlinson GE. ETV6-NTRK3—trk-ing the primary event in human secretory breast cancer. *Cancer Cell*. 2002;2(5):347-348.
17. Tsiatis AC, Norris-Kirby A, Rich RG, et al. Comparison of Sanger sequencing, pyrosequencing, and melting curve analysis for the detection of KRAS mutations: diagnostic and clinical implications. *J Mol Diagn*. 2010;12(4):425-432.
18. Torga G, Pienta KJ. Patient-paired sample congruence between 2 commercial liquid biopsy tests. *JAMA Oncol*. 2018;4(6):868-870.
19. Mok T, Wu YL, Lee JS, et al. Detection and dynamic changes of EGFR mutations from circulating tumor DNA as a predictor of survival outcomes in NSCLC patients treated with first-line intercalated erlotinib and chemotherapy. *Clin Cancer Res*. 2015;21(14):3196-3203.
20. Zill OA, Greene C, Sebisanovic D, et al. Cell-free DNA next-generation sequencing in pancreatobiliary carcinomas. *Cancer Discov*. 2015;5(10):1040-1048.
21. Chae YK, Davis AA, Carneiro BA, et al. Concordance between genomic alterations assessed by next-generation sequencing in tumor tissue or circulating cell-free DNA. *Oncotarget*. 2016;7(40):65364-65373.
22. Thompson JC, Yee SS, Troxel AB, et al. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by next-generation sequencing of cell-free circulating tumor DNA. *Clin Cancer Res*. 2016;22(23):5772-5782.
23. Odegaard JI, Vincent JJ, Mortimer S, et al. Validation of a plasma-based comprehensive cancer genotyping assay utilizing orthogonal tissue- and plasma-based methodologies. *Clin Cancer Res*. 2018;24(15):3539-3549.
24. Heyn H, Esteller M. DNA methylation profiling in the clinic: applications and challenges. *Nat Rev Genet*. 2012;13(10):679-692.
25. Carvalho RH, Haberle V, Hou J, et al. Genome-wide DNA methylation profiling of non-small cell lung carcinomas. *Epigenetics Chromatin*. 2012;5(1):9.
26. Facio FM, Brooks S, Loewenstein J, Green S, Biesecker LG, Biesecker BB. Motivators for participation in a whole-genome sequencing study: implications for translational genomics research. *Eur J Hum Genet*. 2011;19(12):1213-1217.
27. Kaufman D, Murphy J, Scott J, Hudson K. Subjects matter: a survey of public opinions about a large genetic cohort study. *Genet Med*. 2008;10(11):831-839.
28. Kurnit KC, Bailey AM, Zeng J, et al. Personalized cancer therapy: a publicly available precision oncology resource. *Cancer Res*. 2017;77(21):e123-e126.
29. Johnson A, Khotskaya YB, Brusco L, et al. Clinical use of precision oncology decision support [published online September 13, 2017]. *JCO Precision Oncology*. doi:10.1200/PO.17.00036.
30. Meric-Bernstam F, Brusco L, Daniels M, et al. Incidental germline variants in 1000 advanced cancers on a prospective somatic genomic profiling protocol. *Ann Oncol*. 2016;27(5):795-800.
31. Cheng DT, Prasad M, Chekaluk Y, et al. Comprehensive detection of germline variants by MSK-IMPACT, a clinical diagnostic platform for solid tumor molecular oncology and concurrent cancer predisposition testing. *BMC Med Genomics*. 2017;10(1):33.
32. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
33. PMKB. Weill Cornell Medicine. https://pmkb.weill.cornell.edu/. Accessed July 1, 2019.
34. Chakravarty D, Gao J, Phillips, SM, et al. OncoKB: a precision oncology knowledge base [published online May 16, 2017]. *JCO Precis Oncol.* doi:10.1200/PO.17.00011.
35. Beaubier N, Tell R, Lau D, et al. Clinical validation of the tempus xT next-generation targeted oncology sequencing assay. *Oncotarget*. 2019;10(24):2384-2396.
36. CancerPlex. KEW Customer Care. https://kewinc.com/cancerplex-overview/. Accessed July 1, 2019.
37. CancerPlex: Physicians Frequently Asked Questions. CancerPlex, KEW Customer Care, 2017, Retrieved May 9, 2019. kewinc.com/wp-content/uploads/2017/07/Specimen-Handling-Instructions.pdf.
38. Comprehensive tumor profiling. Caris Life Sciences. https://www.carismolecularintelligence.com/comprehensivetumorprofiling. Accessed May 10, 2019.
39. Eifert C, Pantazi A, Sun R, et al. Clinical application of a cancer genomic profiling assay to guide precision medicine decisions. *Per Med*. 2017;14(4):309-325.
40. GPS cancer: molecular insights you can rely on: tumor-normal sequencing of DNA + RNA expression. NantHealth. nanthealth.com/wp-content/uploads/2018/06/GPS-Cancer-Physician-Brochure_20180516_DIGITAL.pdf. Accessed May 10, 2019.
41. Hovelson DH, McDaniel AS, Cani AK, et al. Development and validation of a scalable next-generation sequencing system for assessing relevant somatic variants in solid tumors. *Neoplasia*. 2015;17(4):385-399.
42. MSI added to PCDx NGS panel [press release]. Paradigm. https://www.paradigmdx.com/msi-added-to-pcdx-ngs-panel/. Updated February 21, 2019. Accessed May 10, 2019.
43. FAQ for the OnCORseq NGS TEST. Weill Cornell. http://pathology.weill.cornell.edu/sites/default/files/oncomine_faq.pdf. Accessed May 10, 2019.
44. Oncomine target NGS assays for oncology research. ThermoFisher Scientific. www.thermofisher.com/us/en/home/clinical/preclinical-companion-diagnostic-development/oncomine-oncology.html. Accessed May 10, 2019.
45. Physician FAQ. Paradigm. www.paradigmdx.com/pcdx/physician-faq. Accessed May 8, 2019.
46. Solutions. Guardant Health. guardanthealth.com/solutions/#guardant360. Accessed May 8, 2019.
47. Technical specifications. FoundationOne CDx. https://assets.ctfassets.net/vhribv12lmne/4ZHUEfEiI8iOCk2Q6saGcU/c3361163e2c9bfeb33e934f2d00b0612/F1CDx_Tech_Specs_April_2019_1_.pdf. Accessed May 10, 2019.
48. Technical specifications. FoundationOne Liquid. https://assets.ctfassets.net/vhribv12lmne/3SPYAcbGdqAeMsOqMyKUog/d0eb51659e08d733bf39971e85ed940d/F1L_TechnicalInformation_MKT-0061-04.pdf. Accessed May 15, 2019.
49. Yuki A, Chitwood J, Sidhu H, et al. Analytical validation of the Oncomine™ comprehensive assay v3 with FFPE and cell line tumor specimens in a CAP-accredited and CLIA-certified clinical laboratory [AMP abstract]. *J Molec Diagnostics*. 2018;20(6). https://assets.thermofisher.com/TFS-Assets/CSD/posters/analytical-validation-of-oncomine-comprehensive-assay-v3-in-clinical-lab-poster.pdf