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Clinical Applications of Next-Generation Sequencing in Precision Oncology

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Abstract

The ability of next-generation sequencing to comprehensively assess the molecular profile of a tumor specimen has transformed the clinical testing landscape in oncology. Accordingly, recent years have seen broad uptake of clinical next-generation sequencing to inform cancer patient management. However, significant challenges remain. The annotation and clinical interpretation of variants identified by NGS tests often require rigorous review and may vary between laboratories. While a clearer regulatory path has emerged, reimbursement for NGS tests remains a subject of continuing debate. Basket clinical studies such as NCI-MATCH are evaluating the degree to which matching of a targeted therapy to tumor molecular profile by NGS can be applied independently of tissue histology. Newer applications of NGS such as for circulating tumor DNA testing and to identify novel RNA fusion driver events continue to expand its clinical utility.

Keywords

next-generation sequencing; precision oncology; NCI-MATCH; ctDNA

Since the introduction of commercial next-generation sequencing (NGS) in 2005¹, applications have continued to grow and impact both research and clinical laboratories. Targeted NGS panels are now a mainstay in cancer patient management as are larger whole exome and whole genome sequencing efforts for cancer research. In the last decade, a new generation of cancer therapeutics has emerged that target specific molecular defects found in tumor cells, ushering in the era of precision oncology. As the number of approved targeted treatments continues to grow, NGS test panels are often preferred over single analyte tests because they provide a comprehensive multi-analyte solution that is tissue-conserving.

This review intends to focus on the current state of clinical applications of NGS as related to cancer patient management. It will also discuss some areas in need of attention which if resolved may help to improve the clinical utility of NGS testing. The discussion will cover most aspects of the NGS assay system, including the major testing approaches used and the challenges associated with reporting clinically meaningful variant information that can be readily interpreted by a treating physician. It is noteworthy that at this time many commercial and academic laboratories are offering cancer NGS panels, but no universal

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standards exist for required assay performance and no generally accepted guidelines are available for how the results should be reported. The rapidly evolving regulatory and reimbursement landscape for NGS testing will also be discussed.

Clinical NGS testing overview

Clinical NGS has experienced rapid uptake in recent years, with over 250 academic and commercial Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories offering NGS testing of oncology biospecimens². Most clinical laboratories use targeted gene panels to interrogate a specific set of clinically relevant cancer-related genes. Content may be focused on one or a few histologies that have a high prevalence of clinically actionable mutations, such as lung or colon cancer, but more typically includes several hundred pan-cancer genes such as MSKCC's 468-gene IMPACT test or Foundation Medicine's 359-gene FoundationOne® test. The targeted sequences of interest may be enriched for by either amplification, which uses PCR primers to amplify specific genomic regions, or hybrid capture, which employs oligonucleotides to capture specific regions by hybridization. While amplification-based sequencing offers certain advantages including a generally lower input requirement and faster turn-around times, hybrid capture often gives greater library complexity and uniformity³. Validated assays incorporating both approaches have demonstrated strong performance characteristics on all major variant classes, including single nucleotide variants (SNVs), insertion/deletions (Indels), copy number variants (CNVs) and structural rearrangements such as gene fusions. Notably, for fusions, amplification-based chemistry uses an RNA template to identify breakpoints whereas hybrid capture may use either an RNA template to identify fusion breakpoints or a DNA template to interrogate large intronic regions of clinically relevant fusion driver genes.

In 2015, the Exome Cancer Test (EXaCT-1) became the first New York State-Department of Health-approved whole exome sequencing (WES)-based test for precision oncology⁴. Because the exome is much larger than a targeted panel, EXaCT-1 and other WES assays sequence at substantially lower read depth which translates into a lower sensitivity for variant-calling (i.e. 10 – 20%) than observed with targeted gene panels (i.e. 5 – 10%). Consequently, subclonal resistance mutations or actionable driver alterations present at a low variant allele fraction because of low tumor cellularity may be missed. Additional limitations include the added cost of sequencing a full exome and the generally greater DNA input requirement versus a targeted panel. These considerations, coupled with the ability of targeted panels to accurately assess clinically relevant genomic features such as tumor mutational burden (TMB)⁵, loss of heterozygosity (LOH)⁶, and MSI status^{5,7} without the need for WES has so far limited the use of clinical exome testing.

Some laboratories performing clinical NGS additionally conduct matched germline sequencing, often with DNA from peripheral blood mononuclear cells⁸. The matched germline sequence allows for unequivocal assignment of a variant as somatic versus germline, which has implications for the clinical reporting of NGS results. If a germline variant known to be associated with a hereditary cancer syndrome is identified, that information may potentially be provided to the treating physician who may consider referring the patient and/or patient family members for genetic counseling. Interestingly, the

prevalence of germline cancer alterations in clinically actionable genes appears to be underappreciated and the reporting of these presents an opportunity for more patients and relatives to undergo genetic counseling. In a recent renal cell carcinoma (RCC) study, for example, fully 10% of patients with non-clear cell RCC were found to have germline mutations in genes that were potentially clinically actionable⁹. In a large study of men with metastatic prostate cancer, 11.8% were found to have germline mutations in DNA repair genes, including alterations in *BRCA1*, *BRCA2*, *ATM*, *RAD51D* and *PALB2*, all of which may be amenable to treatment with PARP inhibitors¹⁰. Most of these patients did not have a family history of their cancer type and would have been missed by conventional germline screening guidelines that rely on personal and family history. A final consideration for germline assessment by targeted panels is that it enables the accurate assessment of somatic TMB, an emerging biomarker for immunotherapy.

Variant annotation and clinical interpretation of NGS results

A major remaining challenge for NGS in precision oncology is the annotation and clinical interpretation of variants identified by a laboratory's variant-calling and reporting pipeline, the composition of which may vary substantially from laboratory to laboratory. For SNVs and Indels, many laboratories use a "hotspot"-based approach to identify actionable or clinically relevant variants. The hotspots are at recurrent positions known from Catalog of Somatic Mutations in Cancer (COSMIC) or other cancer gene databases to definitively affect the function of the predicted protein product. Activating alterations in oncogenes such as *EGFR*^{L858R}, *BRAF*^{V600E}, and *KRAS*^{G12D} are well suited to this approach. To supplement the hotspot-based approach, clinical NGS assays additionally use data analysis pipelines that identify alterations predicted to result in truncation of the protein product through the introduction of a termination codon or a frameshift mutation. Such alterations, when they occur in tumor suppressor genes, are generally deleterious or likely deleterious if *in trans* to a loss-of-function event on the opposing allele. However, it may not be possible to know with certainty whether biallelic loss has actually occurred since the opposing allele may be epigenetically regulated or could have been lost due to a large deletion event not interrogated by the NGS panel.

Deciphering how to interpret nucleic acid changes that result in protein missense alterations can be particularly challenging. While algorithms such as SIFT and PolyPhen have been developed to predict *in silico* whether a missense alteration results in a loss or gain of protein function, their specificity is low¹¹. ClinGen has been developed to aid in the clinical interpretation of variants such as these. As stated on its website, ClinGen is "dedicated to building an authoritative central resource that defines the clinical relevance of genes and variants for use in precision medicine and research". In this effort, ClinGen is working in partnership with ClinVar, which is a critical resource for ClinGen. If definitive evidence of pathogenicity or likely pathogenicity does not exist in ClinGen, COSMIC or relevant literature sources, and if the variant has not been previously established to be benign, laboratories should designate the variant as a "variant of uncertain significance". Such variants are not considered to be clinically actionable. Guidelines for the interpretation and reporting of cancer-related variants were published in 2017 by a working group that included

members of the Association of Molecular Pathology (AMP), the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP).¹²

CNVs pose their own unique challenges. Copy number assessment for genes like *MET*, *EGFR* and *ERBB2* has traditionally been performed by FISH. How NGS compares with FISH depends on several factors including the bioinformatics algorithm used to calculate the level of copy number alteration and the tumor cellularity of the sample. Most laboratories use macro- or microdissection to enrich to at least 20% tumor cellularity to mitigate the risk of a false negative result, but enrichment is not always possible. In cases where low tumor cellularity is coupled with a finding of low-level gene amplification that is below the lab's actionable threshold, reflexing to an orthogonal method such as FISH or IHC to confirm the original negative NGS result is an option¹³. A further complication is that some laboratories make a bioinformatic adjustment for tumor cellularity when calculating copy number while others do not^{14,15}. In our own experience, this is a primary source of discordance between NGS platforms. These challenges notwithstanding, both amplification and hybrid-capture target enrichment approaches have demonstrated they can achieve very high concordance when compared with FISH^{13,15,16}. Of note, *ERBB2* merits special attention because therapies targeting *ERBB2* amplifications have proven to be highly efficacious and are FDA-approved in *ERBB2*-amplified/overexpressing breast and gastric/gastroesophageal cancers.¹⁷ While assessment of *ERBB2* amplification by NGS is not yet incorporated into NCCN guidelines, it may only be a matter of time. The ability to perform *ERBB2* testing as part of a comprehensive biomarker assessment using just one NGS test offers clear advantages over sequential testing (e.g. *ERBB2* by IHC, then FISH if the result is equivocal, followed by NGS to identify any other actionable alterations), particularly when tissue is limiting.

After all variants identified in a sample have been annotated, curated knowledgebases are often employed by sequencing laboratories to obtain treatment information about the specific alterations identified. These knowledgebases have been developed specifically for precision oncology and include OncoKB¹⁸, MyCancerGenome¹⁹, CIViC²⁰ and Cancer Genome Interpreter²¹. All emphasize the importance of pre-clinical and clinical data and provide "levels of evidence" to support the use of a targeted therapy for specific identified variants (e.g. an FDA approved drug exists, clinical activity has been observed in clinical trials, or compelling evidence of preclinical activity exists). These knowledgebases thus facilitate clinical decision-making for clinicians and the clinical interpretation of variants by laboratorians preparing a clinical report on their NGS findings.

While knowledgebases like OncoKB can provide a list of FDA-approved targeted therapies for a variant, if any exist, they do not typically provide information about specific clinical trials that might be considered for potentially actionable variants where an FDA-approved drug is not yet available. Third party vendors such as N-of-One, PerianDx and OncoPrint Knowledge Reporter fill this void by providing up-to-date clinical trials annotation for actionable variants. In our experience, they also can research and annotate the clinical relevance of a novel variant that may not be in existing knowledgebases, usually with a turnaround time of less than one day. Ultimately, a publicly available knowledge resource, updated regularly and cataloging definitive information about the clinical actionability of variants in cancer-related genes while linking this information to FDA-approved therapies or

eligible clinical trials, would be of immense value to oncologists and their patients. Of note, a collaborative group known as the Variant Interpretation for Cancer Consortium (VICC) has recently undertaken an effort to standardize and integrate information from multiple knowledgebases, including OncoKB and CIViC, into a single interface for public use.²² Relevant clinical trial information will also be incorporated into this database.

Regulatory and reimbursement landscape for clinical NGS

The vast majority of NGS clinical assays are laboratory developed tests (LDTs). FDA considers LDTs to be in vitro diagnostic (IVD) tests that are “designed, manufactured and used within a single laboratory”²³. Historically, FDA has exercised enforcement discretion over LDTs and these tests have instead fallen under the regulatory purview of the Center for Medicare and Medicaid Services (CMS). Under CMS, clinical NGS tests must be appropriately validated according to Clinical Laboratory Improvement Amendments (CLIA) regulations and testing must be performed in a CLIA-certified laboratory.

Recently, FDA has begun to define a regulatory path for the approval or clearance of NGS tests. In late 2017, FDA and CMS established a novel process, designated the CMS-FDA Parallel Review Program, by which both agencies would concurrently review an NGS or other medical device. The new parallel review process will help decrease the time between FDA approval and a decision by CMS to provide national coverage through a national coverage determination (NCD) for an NGS clinical test²⁴.

Using the CMS-FDA Parallel Review Program, Foundation Medicine’s FoundationOne CDx (F1CDx) received FDA approval as an IVD test, the first for NGS²⁵. At one level the test is a companion diagnostic (CDx) that can be used to assign a patient to any of 15 different FDA-approved targeted therapies in five different tumor types. This represented a breakthrough in FDA’s approach to companion diagnostics, which previously had matched one biomarker test to one drug. Additionally, the FDA stated in their press release that any genetic aberrations identified by the 324-gene F1CDx test “can be used by physicians according to professional guidelines to manage cancer patients”.

Concurrent with FDA approval of F1CDx, Memorial Sloan Kettering Cancer Center’s Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) NGS assay used a slightly different approach and received FDA marketing “authorization” using the De Novo 510(k) review pathway. FDA also established a novel regulatory pathway for NGS assays by accrediting the New York State Department of Health (NYSDOH), which had previously reviewed and approved IMPACT, as a third-party reviewer for future 510(k) submissions²⁶. Future applicants can therefore submit an application to the FDA directly or through NYSDOH.

Additional approvals have since been awarded to a growing list of other NGS tests. These include Illumina’s Praxis Extended RAS Panel, Thermo Fisher Scientific’s Oncomine Dx Target Test and FoundationFocus CDxBRCA (Table 1). Of interest, after FDA approved FoundationFocus CDxBRCA as a CDx, it later approved FoundationFocus™ CDx BRCA LOH as a complementary diagnostic to assess for homologous recombination

deficiency status in ovarian cancer patients being considered for maintenance therapy with the PARP inhibitor rucaparib²⁷. In keeping with the designation of complementary diagnostic, FoundationFocus™ CDx *BRCA* LOH may identify patients more likely to respond to rucaparib but testing is not a prerequisite for treatment.

Moreover, NGS IVD submissions have not been limited to tumor testing. In the fall of 2018, FDA granted 510(k) clearance to Adaptive Biotechnologies' ClonoSEQ, an NGS-based assay for the detection of minimal residual disease in patients with acute lymphoblastic leukemia (ALL) or multiple myeloma²⁸. Foundation Medicine, Guardant Health, Memorial Sloan Kettering Cancer Center and Personal Genome Diagnostics have all announced they are working with FDA to garner approval or clearance for their NGS-based liquid biopsy tests. Collectively, these developments are game-changing and reflect growing acceptance of the use of NGS tests as part of the standard-of-care for patients with cancer.

Reimbursement for NGS clinical testing has been the subject of an ongoing debate between clinicians, patients, insurers and regulators. CMS has stated that tests with FDA approval, such as F1CDx, or with 510(k) clearance, such as MSK-IMPACT, will have national coverage. However, the majority of NGS test developers are at smaller institutions without FDA-cleared or approved tests. CAP and the American Clinical Laboratory Association among others have expressed concern that these tests, lacking FDA approval or clearance, might not be covered by Medicare, the nation's largest health care insurer, or by private insurers²⁹. To address the concern, CMS provided a path for local coverage determination through CMS's Medicare Administrative Contractors (MACs). However, it remains unclear how local coverage decisions will be made and the extent to which individual NGS clinical tests will be reimbursed by MACs. Recently, amid concerns that CMS' current NCD policy would limit coverage for genetic testing in genes like *BRCA1* or *BRCA2* to only patients with late stage disease, CMS has agreed to reopen its coverage guidelines for public comment and potential revision to ensure these tests are appropriately covered.³⁰

After a testing laboratory generates NGS test results for a patient and issues a clinical report to the treating physician, there is no comprehensive effort to track what drug was administered and the ultimate clinical outcome. Tracking such data would comprehensively inform the clinical utility of precision oncology, particularly for rare tumor histologies or in cases of off-label use of targeted therapies where clinical trials data are sparse²⁹. Ideally, these data would be collected in a national registry in a way that would not be overly burdensome. Data from such a registry would provide important support for reimbursement of NGS testing by MACs and private insurers.

NCI-MATCH: A precision oncology trial with patient selection based on NGS

NGS testing is now being used to support large "basket" clinical trials for precision oncology. Basket studies use genomic testing to direct patients to a targeted therapy that matches their molecular profile, independent of tumor histology. Prominent recent and ongoing basket studies include Targeted Agent and Profiling Utilization Registry (TAPUR) ()³¹, MyPathway ()³², and Molecular Analysis of Therapy Choice (NCI-MATCH) ()³³.

Among these, NCI-MATCH is the largest and most elaborate. Activated in August 2015, NCI-MATCH will have opened 39 separate trial arms before the end of 2019 at approximately 1100 clinical trial sites throughout the United States.

A novel aspect of the NCI-MATCH study framework was the establishment of a central laboratory network for the molecular screening of patients by NGS (Figure 1). The central lab network performed NGS testing on over 6000 patients. Our laboratory at Frederick National Laboratory for Cancer Research (FNLCR) worked with Thermo Fisher Scientific to develop the central NGS assay, OncoPrint Comprehensive Assay (OCA), and set up a network of five laboratories including MD Anderson Cancer Center (MDACC), Massachusetts General Hospital (MGH), Yale University School of Medicine (YSM), Dartmouth Hitchcock Medical Center (DHMC), and ourselves (FNLCR). The laboratory network provided redundancy when individual labs had to suspend testing to version the assay or for other reasons. Additionally, distribution of samples across the lab network enabled the efficient management of very high testing loads experienced by NCI-MATCH, particularly in the early phase of the trial. A critical element was that all laboratories used a validated and harmonized assay workflow featuring a common set of standard operating procedures, highly similar instrumentation, and a uniform data analysis pipeline. Indeed, prior to study start an inter-laboratory reproducibility study was conducted in which a positive pairwise concordance of >96% for DNA variants and 100% for RNA fusions was achieved¹⁴. The reproducibility study demonstrated that, despite the complexity of the test system, high inter-laboratory reproducibility of NGS data is possible when a common workflow is used.

A contemporaneous “fresh” biopsy was obtained from each of the first ~6000 patients screened by the central NGS assay. Fully 93% of patients had an evaluable NGS result (unpublished data), which compares favorably to NGS success rates from other published studies³⁴. The high success rate can in part be attributed to the additional trial requirement for a fine needle aspirate (FNA) where possible. In 7% of cases, a tumor specimen was not evaluable due to low tumor cellularity, but the sequencing result was rescued by the matched FNA. Additionally, the OCA sequencing platform with its low input requirement (20 ng DNA and 20 ng RNA) further contributed to the high success rate. Interestingly, NCI-MATCH investigators are now conducting a correlative study to evaluate the extent to which primary archival specimens have the same actionable mutation as was identified in the corresponding fresh biopsy used to inform NCI-MATCH treatment. This study may identify circumstances where NGS testing of an archival specimen would be sufficient to accurately inform the molecular profile and clinical management of a patient.

In May of 2017 NCI-MATCH transitioned to a new screening approach using outside “designated labs” to fill the remaining 19 NCI-MATCH arms still accruing patients, as well as any newly opened arms going forward (Figure 2). In the new paradigm, the role for the NCI-MATCH central assay is to confirm all variants identified by the designated labs. As of April, 2019, 23 laboratories have been admitted to the NCI-MATCH designated laboratory network and more are still being evaluated. The final step of the application process requires all applicants to undergo a qualification test. Here, DNA from eight cell lines and two clinical samples is sent to each candidate lab and sequencing results are compared to those

obtained with the NCI-MATCH central assay. The use of DNA in the qualification testing removes pre-analytical variables such as tumor heterogeneity. Furthermore, only those regions that are common to both the designated lab and NCI-MATCH test panels are evaluated. Incorporating these two elements into the study design has contributed to the high mean concordance we have so far observed between outside lab and NCI-MATCH assays of >95% positive percent agreement for reportable SNVs, indels and CNVs³⁵. It remains to be seen how the high concordance rate observed in the qualification study will translate to real-world NCI-MATCH testing and central confirmation.

Emerging NGS assays and technologies

ctDNA

In a few short years, the assessment of circulating tumor DNA (ctDNA) from plasma has emerged as a powerful complement to tumor biopsies for the clinical management of patients with cancer. Liquid biopsies are minimally invasive, can generally be obtained at any time during treatment to allow for real-time assessment of the tumor molecular landscape, and may capture molecular heterogeneity missed by tumor biopsy due to sampling only a small fraction of the total tumor burden. The field still has a “Wild West” element, with a growing list of commercial and academic laboratories offering clinical testing services. The largest entity among these is Guardant Health, which has performed testing on more than 80,000 patients with cancer as of January 2019³⁶.

The greatest clinical potential for ctDNA may be in informing a treatment decision without the need for a tumor biopsy. However, other potential clinical applications have generated intense interest. These include prediction of therapy response based on early ctDNA dynamics, monitoring for minimal residual disease after surgical resection in early/mid-stage cancer patients, serial monitoring for emergence of resistance to therapy, and early detection of cancer³⁷. A recent article suggested that outside of *EGFR* in lung cancer and *KRAS* in colorectal cancer there is limited evidence of true clinical validity for ctDNA assays³⁸. Several groups are currently conducting prospective studies with ctDNA biomarkers interrogated on NGS-based platforms to address this^{39,40}.

A primary consideration for the development of NGS clinical assays for ctDNA is the exquisite sensitivity required, which must not come at the expense of specificity. The median allele fraction of somatic variants typically identified in ctDNA, even in late-stage patients with high tumor burden, is substantially less than 1%⁴¹. This requires an assay at least an order of magnitude more sensitive than most tumor NGS targeted gene panels. To achieve this, special strategies have been devised for ctDNA which include the incorporation of unique molecular indices on each molecule captured, very deep sequencing (e.g. raw read depths > 20,000 reads at each target region), and error correction algorithms that remove sequencing artifacts introduced at very low levels during nucleic acid extraction, library preparation and sequencing.

Almost all clinical sequencing performed today for ctDNA employs targeted panels comprised of less than 100 genes. Examples include Guardant360® (73 genes), FoundationAct® (60 genes), PlasmaSELECT™64 from Personal Genome Diagnostics (64

genes) and Roche AVENIO ctDNA expanded kit (77 genes). This is substantially smaller than most tumor NGS targeted panels, which typically interrogate hundreds of cancer-related genes. Given the much greater sequencing depth required for ctDNA versus tumor testing, a smaller panel is required to keep costs manageable. However, recent developments may be paving the way for adoption of much larger gene panels. Of keen interest for immunotherapy studies is the assessment of TMB in plasma. Two recent retrospective analyses suggest that plasma TMB is predictive of clinical outcomes in patients treated with immune checkpoint inhibitors^{42,43}. Both studies used large gene panels covering more than 1 Mb, consistent with data suggesting that larger gene panels of at least 500 kb are likely required for accurate assessment of TMB⁵. Another advantage of larger panels is that they are better suited to early cancer detection or monitoring for minimal residual disease than smaller panels. For these applications, casting a wide net increases the odds of finding traces of nascent or re-emerging disease in the blood. One company, GRAIL, is using a 500-gene ctDNA panel in a quest to identify cancer in its earliest stages. Interestingly, it reported clonal hematopoiesis (CH) in over 30% of healthy donor subjects it tested over the age of 70⁴⁴. CH results from the expansion of a hematopoietic stem cell clone that has acquired a somatic alteration conferring a selective advantage. CH variants will therefore appear in cell-free DNA (cfDNA) and could be confused with somatic variants of tumor origin. While the large majority of CH alterations do not appear to be clinically actionable⁴⁴, an ongoing debate is whether deep sequencing of a matched white blood cell fraction is needed with every cfDNA analysis to definitively identify any somatic alterations that may have resulted from CH.

RNA as target

Cancer fusion events—This review has been focused on DNA as NGS target. Indeed, the clinical utility of identifying actionable DNA-based genomic alterations for patient management has been repeatedly demonstrated. However, there is growing appreciation that RNA sequencing data may complement DNA in the clinical management of patients with cancer. One example is the identification of fusion events using an RNA template. Therapeutically actionable RNA fusions have been identified in multiple carcinoma types⁴⁵ and are common in hematological cancers and sarcomas where their identification is especially helpful for diagnosis or prognosis. In our laboratory we use an RNA targeted sequencing approach to interrogate over 800 known fusion events in 51 cancer driver genes with the OncoPrint Comprehensive Assay. Other targeted RNA sequencing technologies, such as that from ArcherDx, are agnostic to the fusion partner and thus enable the identification of novel fusion events. By comparison, many targeted DNA panels interrogate a more limited number of fusion drivers, if any, because of the requirement to include large intronic regions to identify breakpoints. An additional advantage for RNA compared to DNA is that many fusion driver events are over-expressed in cancer and may thus be more readily identified using RNA as template.

T cell-inflammation signature—Recently several high impact, retrospective studies reported that a gene expression profile (GEP) representative of a T cell-inflamed tumor microenvironment may enrich for response to immune checkpoint blockade in multiple tumor types⁴⁶⁻⁴⁸. Moreover, the T-cell inflamed GEP may complement the assessment of

tumor mutational burden in the identification of patients most likely to respond to anti-PD-1/PD-L1 therapy. Further validation is needed, but the data are compelling enough that future prospective anti-PD-1/PD-L1 clinical trials may use a tumor inflammation signature as selection or stratification biomarker. The T cell inflamed signature identified consisted of 18 genes and was developed on the Nanostring nCounter® system. However, other similar signatures have been identified which are highly correlated with the Nanostring signature⁴⁶ and thus could potentially be used in lieu of it. These could readily be incorporated into an RNA targeted sequencing panel.

T cell receptor sequencing—Still another emerging area of interest for immunotherapy is the profiling of the T cell receptor (TCR) repertoire. Several studies suggest that the assessment of TCR clonality at baseline and the monitoring of TCR clonal expansion during treatment may be predictive of response to immune checkpoint blockade⁴⁹⁻⁵¹. The complementarity determining region 3 (CDR3) of the T cell receptor β chain, which encodes a highly variable region of the TCR in direct contact with peptide antigen, is a common target of TCR sequencing assays. Use of an RNA versus a genomic DNA template is preferred as the presence of multiple RNA copies facilitates the identification of individual TCR clones. Although there are currently no clinical applications for TCR sequencing, there is intense interest to further explore its potential clinical utility.

Summary

In a few short years, clinical NGS testing has begun to redefine the way that treating physicians manage patients with cancer. However, for all its successes, significant challenges remain. For example, while knowledgebases such as OncoKB have been developed to help assess the clinical actionability of variants in cancer-related genes, ambiguous cases will invariably be encountered. How should these be reviewed and reported? An additional challenge relates to bringing NGS testing to underserved patient populations. The recent clearance/approval of several NGS assays and the national coverage determination that accompanied them has brought hope of broad access to these tests, but there are lingering concerns that underserved patient populations will continue to have limited access. Notably, the National Cancer Institute is in the late stages of planning for the Cancer Moonshot Biobank, which aims to bring clinical NGS testing to diverse populations served by community hospitals. Despite the challenges, we believe NGS clinical testing and precision oncology in general have improved the lives of patients with cancer. In support of this, there have been several publications highlighting the improved clinical outcomes associated with treating patients based on a targeted alteration identified by NGS⁵². With the more recent addition of liquid biopsies to the test armamentarium, NGS will continue to transform the management of cancer patient care for years to come.

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REFERENCES

1. Kulkarni JK. Next-Generation Sequencing — An Overview of the History, Tools, and “Omic” Applications In: Kulkarni JK, ed. Next Generation Sequencing - Advances, Applications and Challenges. IntechOpen 2016.
2. Wadapurkar RM, Vyas R. Computational analysis of next generation sequencing data and its applications in clinical oncology. Informatics in Medicine Unlocked 2018;11:75–82.
3. Samorodnitsky E, Jewell BM, Hagopian R, et al. Evaluation of Hybridization Capture Versus Amplicon-Based Methods for Whole-Exome Sequencing. Hum Mutat 2015;36:903–14. [PubMed: 26110913]
4. Rennert H, Eng K, Zhang T, et al. Development and validation of a whole-exome sequencing test for simultaneous detection of point mutations, indels and copy-number alterations for precision cancer care. NPJ Genom Med 2016;1.
5. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9:34. [PubMed: 28420421]
6. Swisher EM, Lin KK, Oza AM, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. Lancet Oncol 2017;18:75–87. [PubMed: 27908594]
7. Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. Cancer medicine 2018;7:746–56. [PubMed: 29436178]
8. Hyman DM, Taylor BS, Baselga J. Implementing Genome-Driven Oncology. Cell 2017;168:584–99. [PubMed: 28187282]
9. Carlo MI, Mukherjee S, Mandelker D, et al. Prevalence of Germline Mutations in Cancer Susceptibility Genes in Patients With Advanced Renal Cell Carcinoma. JAMA Oncol 2018;4:1228–35. [PubMed: 29978187]
10. Pritchard CC, Mateo J, Walsh MF, et al. Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. N Engl J Med 2016;375:443–53. [PubMed: 27433846]
11. Flanagan S, Patch AM, Ellard, J. Using SIFT and PolyPhen to Predict Loss-of-Function and Gain-of-Function Mutation. GENETIC TESTING AND MOLECULAR BIOMARKERS 2010;14.
12. Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn 2017;19:4–23. [PubMed: 27993330]
13. Ross DS, Zehir A, Cheng DT, et al. Next-Generation Assessment of Human Epidermal Growth Factor Receptor 2 (ERBB2) Amplification Status: Clinical Validation in the Context of a Hybrid Capture-Based, Comprehensive Solid Tumor Genomic Profiling Assay. J Mol Diagn 2017;19:244–54. [PubMed: 28027945]
14. Lih CJ, Harrington RD, Sims DJ, et al. Analytical Validation of the Next-Generation Sequencing Assay for a Nationwide Signal-Finding Clinical Trial: Molecular Analysis for Therapy Choice Clinical Trial. J Mol Diagn 2017;19:313–27. [PubMed: 28188106]
15. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013;31:1023–31. [PubMed: 24142049]
16. Lih CJ, Sims DJ, Harrington RD, et al. Analytical Validation and Application of a Targeted Next-Generation Sequencing Mutation-Detection Assay for Use in Treatment Assignment in the NCI-MPACT Trial. J Mol Diagn 2016;18:51–67. [PubMed: 26602013]
17. Meric-Bernstam F, Johnson AM, Dumbrava EEI, et al. Advances in HER2-Targeted Therapy: Novel Agents and Opportunities Beyond Breast and Gastric Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research 2019;25:2033–41. [PubMed: 30442682]
18. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: A Precision Oncology Knowledge Base. JCO Precis Oncol 2017;2017.

19. Taylor AD, Micheel CM, Anderson IA, Levy MA, Lovly CM. The Path(way) Less Traveled: A Pathway-Oriented Approach to Providing Information about Precision Cancer Medicine on My Cancer Genome. *Translational oncology* 2016;9:163–5. [PubMed: 27084433]
20. Griffith M, Spies NC, Krysiak K, et al. CIViC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. *Nature Genetics* 2017;49:170. [PubMed: 28138153]
21. Tamborero D, Rubio-Perez C, Deu-Pons J, et al. Cancer Genome Interpreter annotates the biological and clinical relevance of tumor alterations. *Genome Med* 2018;10:25. [PubMed: 29592813]
22. The Variant Interpretation for Cancer Consortium (VICC). 2019,
23. U.S. Food & Drug Administration. Laboratory Developed Tests. <https://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/laboratorydevelopedtests/default.htm>2018.
24. Jean NS, Pinto C, Tenente I, Murray G. Collaboration is key to accelerating diagnostics access to optimize benefits of precision medicines. *Personalized medicine* 2018;15:157–61. [PubMed: 29843546]
25. U.S. Food & Drug Administration. FDA announces approval, CMS proposes coverage of first breakthrough-designated test to detect extensive number of cancer biomarkers. <https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm587273.htm>: U.S. Food and Drug Administration; 2017.
26. U.S. Food & Drug Administration. FDA unveils a streamlined path for the authorization of tumor profiling tests alongside its latest product action. <https://www.fda.gov/newsevents/newsroom/pressannouncements/ucm585347.htm>: U.S. Food and Drug Administration; 2017.
27. U.S. Food & Drug Administration. FoundationFocus CDxBRCA LOH - P160018/S001. <https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm605446.htm>2018.
28. U.S. Food & Drug Administration. FDA authorizes first next generation sequencing-based test to detect very low levels of remaining cancer cells in patients with acute lymphoblastic leukemia or multiple myeloma. In: Caccamo S, ed. <https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm622004.htm>: U.S. Food & Drug Administration; 2018.
29. Ray T CMS Final Coverage Policy for NGS Cancer Panels Eases Some Lab Industry Concerns. 3 19, 2018 ed. <https://www.genomeweb.com/sequencing/cms-final-coverage-policy-ngs-cancer-panels-eases-some-lab-industry-concerns#.XMMiSzBKiw4:genomeweb>.
30. CMS to Revisit National Coverage Determination on NGS Tumor Testing. 2019 at <https://www.genomeweb.com/molecular-diagnostics/cms-revisit-national-coverage-determination-ngs-tumor-testing#.XMbeRTBKipo>.)
31. Mangat PK, Halabi S, Bruinooge SS, et al. Rationale and Design of the Targeted Agent and Profiling Utilization Registry Study. 2018:1–14.
32. Hainsworth JD, Meric-Bernstam F, Swanton C, et al. Targeted Therapy for Advanced Solid Tumors on the Basis of Molecular Profiles: Results From MyPathway, an Open-Label, Phase IIa Multiple Basket Study. *J Clin Oncol* 2018;36:536–42. [PubMed: 29320312]
33. NCI-MATCH / EAY131. 2019 at <https://ecog-acrin.org/nci-match-eay131>.)
34. Goswami RS, Luthra R, Singh RR, et al. Identification of Factors Affecting the Success of Next-Generation Sequencing Testing in Solid Tumors. *American journal of clinical pathology* 2016;145:222–37. [PubMed: 27124905]
35. Tricoli J, Zane L, Harrington RD, et al. Design and Development of the Molecular Analysis for Therapy Choice (NCI-MATCH) Designated Laboratory Network. 55th annual American Society for Clinical Oncology meeting Chicago, Illinois 2019.
36. Guardant Health Inc. Guardant Health Announces Launch of the LUNAR Assay for Detection of Early-Stage Cancer and Recurrence to Academic and Biopharmaceutical Researchers. In: Wein JS, Ian., ed. GlobalNewswire: Guardant Health, Inc; 2019.
37. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer* 2017;17:223–38. [PubMed: 28233803]

38. Merker JD, Oxnard GR, Compton C, et al. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. *J Clin Oncol* 2018;36:1631–41. [PubMed: 29504847]
39. Guardant Health Providing Liquid Biopsy for Japanese GI Cancer Basket Trial genomeweb. 3 13, 2018, at <https://www.genomeweb.com/cancer/guardant-health-providing-liquid-biopsy-japanese-gi-cancer-basket-trial#.XMMi5jBKiw5>.)
40. Mok T, Gadgeel S, Kim ES, et al., Blood first line ready screening trial (B-FIRST) and blood first assayscreening trial (BFAST) enable clinical development of novel blood-based biomarker assays for tumor mutational burden (TMB) and somatic mutations in 1L advanced or metastatic NSCLC. ESMO 2017 Congress Madrid, Spain 2017.
41. Lanman RB, Mortimer SA, Zill OA, et al. Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. *PLoS one* 2015;10:e0140712. [PubMed: 26474073]
42. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med* 2018;24:1441–8. [PubMed: 30082870]
43. Guardant Health Inc. GuardantOMNI (500-gene panel) bTMB and Response to Immunotherapy ESMO; 2018; Munich, Germany.
44. Razavi P, Li BT, Hou C, et al. Cell-free DNA (cfDNA) mutations from clonal hematopoiesis: Implications for interpretation of liquid biopsy tests. 2017;35:11526-.
45. Mertens F, Johansson B, Fioretos T, Mitelman F. The emerging complexity of gene fusions in cancer. *Nat Rev Cancer* 2015;15:371–81. [PubMed: 25998716]
46. Cristescu R, Mogg R, Ayers M, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science (New York, NY)* 2018;362.
47. Ott PA, Bang YJ, Piha-Paul SA, et al. T-Cell-Inflamed Gene-Expression Profile, Programmed Death Ligand 1 Expression, and Tumor Mutational Burden Predict Efficacy in Patients Treated With Pembrolizumab Across 20 Cancers: KEYNOTE-028. *J Clin Oncol* 2019;37:318–27. [PubMed: 30557521]
48. Ribas A, Robert C, Schacter J, et al., Tumor Mutational Burden, T Cell-inflamed Gene Expression Profile and PD-L1 Are Independently Associated With Response to Pembrolizumab in Patients With Advanced Melanoma in the KEYNOTE-006 Stud. AACR Annual Meeting 2019 Atlanta, Georgia 2019.
49. Hopkins AC, Yarchoan M, Durham JN, et al. T cell receptor repertoire features associated with survival in immunotherapy-treated pancreatic ductal adenocarcinoma. *JCI Insight* 2018;3.
50. Robert L, Tsoi J, Wang X, et al. CTLA4 blockade broadens the peripheral T-cell receptor repertoire. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2014;20:2424–32. [PubMed: 24583799]
51. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;515:568–71. [PubMed: 25428505]
52. Morash M, Mitchell H, Beltran H, Elemento O, Pathak J. The Role of Next-Generation Sequencing in Precision Medicine: A Review of Outcomes in Oncology. *Journal of personalized medicine* 2018;8.

FIGURE 1.

NCI-MATCH test workflow for the first ~6000 patients screened. Each patient tested through the central laboratory network had their sequencing results compiled into a clinical report that was sent to the treating physician at the clinical site. Results from clinical reports were discussed with and provided to the patient. Sequencing results were also uploaded by the network laboratory into MATCHBox, a rules engine that assigned eligible patients to a treatment arm. All treatment assignments were reviewed by a group of clinical and scientific experts before final sign-off. MDACC indicates MD Anderson Cancer Center; FNLCR, Frederick National Laboratory for Cancer Research; MGH, Massachusetts General Hospital; YSM, Yale University School of Medicine; DHMC, Dartmouth Hitchcock Medical Center; ECOG-ACRIN, Eastern Cooperative Oncology Group (ECOG) and the American College of Radiology Imaging Network (ACRIN).

FIGURE 2.

NCI-MATCH test workflow for the designated laboratory network. The designated lab reviews test reports and notifies NCI-MATCH clinical sites of patients potentially eligible for NCI-MATCH. If the patient is registered by the clinical site, the designated lab receives an email notification to upload the patient's variant data for central review. After the patient is assigned to treatment, a specimen is also sent to the central laboratory network for confirmation of the actionable variant identified by the designated lab.

TABLE 1.

FDA-cleared or approved NGS oncology tests

Test	Company or Institution	FDA Status	Description
MSK-IMPACT	Memorial Sloan Kettering Cancer Center	Cleared	468-gene matched tumor and normal test intended to provide information on somatic mutations and microsatellite instability for use in accordance with professional guidelines
FoundationOne CDx	Foundation Medicine	Approved	324-gene test that also assesses TMB and MSI. Can be used to assign a patient to 15 different targeted therapies in 5 different tumor types. It provides mutational information that may help in the clinical management of cancer patients.
FoundationFocus CDx/ <i>BRCA</i>	Foundation Medicine	Approved	For the qualitative detection of <i>BRCA1</i> and <i>BRCA2</i> alterations in FFPE ovarian tumor tissue
Oncomine Dx Target Test	Thermo Fisher Scientific	Approved	A companion diagnostic to identify <i>ROS1</i> fusions, <i>BRCA1</i> ^{N600E} mutations and <i>EGFR</i> ^{L858R} mutations or <i>EGFR</i> ^{Ex 19del} in NSCLC patients. Can detect alterations in 23 genes in tumor from NSCLC patients.
ClonoSEQ	Adaptive Biotechnologies	Cleared	For the detection and monitoring of minimal residual disease from patients with multiple myeloma and B-cell acute lymphoblastic leukemia
Praxis Extended RAS Pane	Illumina	Approved	A companion diagnostic for panitumumab. Detects the presence of 56 specific mutations in <i>RAS</i> genes (<i>KRAS</i> exons 2, 3, and 4 and <i>NRAS</i> exons 2, 3, and 4) in the tumor tissue of patients with mCRC

TMB indicates tumor mutational burden; MSI indicates microsatellite instability; FFPE indicates formalin-fixed paraffin embedded; NSCLC indicates non-small cell lung cancer; mCRC indicates metastatic colorectal cancer