EMERGING TECHNOLOGIES AND THERAPEUTICS REPORT

Genomic Sequencing to Guide Cancer Management
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• This review of commercially available next-generation sequencing (NGS) tests for cancer management includes 321 published clinical research studies reporting on a variety of outcome measures, including identification of actionable mutations, patient management decisions, and patient health outcomes.

• Most published studies analyzed single groups of patients who underwent genomic testing, and most of these reported on the number or percentage of patients with actionable mutations. Fewer studies reported on treatment decisions or patient health outcomes.

• Only about 5% of studies compared health outcomes in patients managed with genomic testing vs patients managed without testing. Some of these studies provide evidence for positive impacts of NGS tests on cancer treatment, but they are generally of low methodologic quality (eg, cohort studies or single-group studies with post hoc comparisons). None were randomized controlled trials. Evidence comparing the impact of multigene panel tests with alternative strategies such as serial single-gene testing is even more limited.

• Broader applications of NGS technology, such as whole-exome and whole-genome sequencing, presently used mostly for research, will likely have increasing impact on precision medicine in coming years.

• Significant disparities exist in access to genomic sequencing technology for underserved patient populations as well as clinician training on these technologies in small community and rural treatment settings. Effort will be required to ensure this technology’s benefits are available to all patients.

Summary of Findings

Advances in cancer management, primarily in the form of recently developed targeted drug therapies, depend on companion diagnostic (CDx) tests that identify gene variants to inform the best candidate patients for a particular drug therapy. The number of companion diagnostic tests and associated therapies, and their application to different cancer types, has continued to increase over the past 2 decades. Over this same period, next-generation sequencing (NGS) technology, which allows many genes to be sequenced at once, has evolved rapidly and is now in mainstream clinical use. NGS-based tests can provide a more comprehensive assessment of a cancer’s genomic profile. The technology and its applications have driven an increase in the diversity and complexity of commercially available tests for mainstream clinical use.

In response to these rapid changes in cancer care, the Patient-Centered Outcomes Research Institute (PCORI) commissioned this emerging technology report to ascertain the landscape of clinical evidence documenting the impact of commercially available NGS-based multigene panel tests on the management and health outcomes of patients with cancer, what is commonly termed clinical utility. We approached this task using the technique of evidence mapping, a form of systematic methodology for summarizing the quantity and quality of evidence underlying a broad topic or field of study. A major objective of evidence
mapping is to identify areas or topics for which evidence is lacking (termed evidence gaps) and to summarize these findings in a visually convenient format.

We reviewed published clinical literature from January 2010 through August 2020. We selected studies reporting on data pertaining to clinical utility, including the number of clinically actionable gene variants (those with potential for influencing patient care) and patient health outcomes, including overall and progression-free survival. We also examined studies reporting on the comparative performance of liquid biopsy tests that use blood samples rather than standard solid tissue specimens as well as the use of newer, still-emerging NGS technologies in clinical care. We also reviewed gray literature, policies from the Centers for Medicare & Medicaid Services (CMS), local coverage determinations from CMS providers, and recent clinical trials pertaining to this subject. To inform the project’s scope and content, we conducted semistructured interviews with a diverse set of stakeholders, including clinicians, payers, public policymakers, patient advocates, and patients, to garner their input on the major issues surrounding NGS technology and its application to managing care for patients with cancer.

Our literature search identified 4913 potentially relevant citations. We selected 945 eligible studies reviewed in depth through examination of full-text published articles and ultimately identified 321 published studies that met our inclusion criteria. Our review revealed very limited evidence supporting the benefit of widely available commercial NGS-based genomic tests for cancer management. Study designs deemed of greatest methodologic value for this purpose, such as randomized controlled trials, were not found. Few studies (n = 15; about 5%) provided comparisons of patients managed with genomically guided treatment vs those managed without genomic testing. These studies were observational in design: either comparative cohort studies or single-group studies with post hoc comparisons.

The evidence base consisted mostly of retrospective, noncomparative observational studies. Because these studies lack data comparing use of NGS testing with standard patient management strategies, they cannot provide rigorous evidence for clinical utility. Further, most of these studies report the number of clinically actionable gene mutations identified by NGS tests but do not report patient management decisions and health outcomes resulting from management guided by testing. Moreover, tests from 2 commercial laboratories were frequently used in the studies we reviewed; less data exist for tests from other laboratories. Additionally, our searches revealed a paucity of information for most cancer types; lung cancer is the most extensively studied tumor type, and most studies enrolled a mix of patients with different cancer types.

The scarcity of studies comparing patients managed with genomic testing vs those managed with standard care constitutes a major evidence gap for formally assessing genomic sequencing’s impact to guide patient management. The shortage of such evidence suggests that high-quality studies may be difficult or impractical to conduct. If so, then current evidence gaps may persist.

One potential way to address this limitation is to incorporate indirect evidence for clinical utility. For instance, by quantifying the number of genetic variants associated with clinical benefit (such as biomarkers assayed by US Food and Drug Administration–approved companion diagnostic tests) that are included in an NGS panel, a chain of evidence can be constructed to support its clinical value. A crucial component of such a strategy would be to demonstrate that an NGS panel accurately and reliably detects these individual biomarkers (ie, has adequate analytic validity). Emphasis on individual biomarkers could facilitate research into other evidence gaps, such as (1) evaluating the analytic validity of newer liquid biopsy tests and (2) performing a more critical and systematic examination of actionability, the potential
for clinical impact, for individual biomarkers that are backed by less evidence. This latter consideration is particularly important because criteria for actionability vary across individual studies and are often unspecified. Standardized approaches to defining, validating, and tracking actionable variants and associated treatment options in a rigorous and fully transparent manner may help clarify the overall clinical value of multigene panel tests and facilitate direct comparison of results from different studies. Many ongoing efforts address this goal, and we briefly review some examples in our discussion.

Targeted multigene panels, which analyze a select set of genes with known relevance to the condition of interest, are the most extensively used NGS technologies for managing patients with cancer. While these tests are currently the overwhelming choice for treatment selection, larger-scale technologies, such as whole-exome sequencing (WES) and whole-genome sequencing (WGS), are gaining traction in research settings. These technologies have potential to identify many more pathogenic genetic variants compared with targeted testing, but this gain may be accompanied by reduced accuracy. Also, these larger-scale tests can increase the time required to generate and interpret results, and the informational burden (ie, the number and diversity of detected variants) may introduce decisional uncertainty for clinicians. Research on multiomic tests—comprehensive analyses incorporating WES or WGS as well as RNA sequencing, gene expression profiling, or methylation analysis—is also increasing, but broad adoption will likely require identification of key predictive markers with clinical validity to drive uptake. While additional research will be required to properly leverage these technologies to benefit patients, these technologies will likely be used in routine clinical care in the near future.

Analyzing differences in a cancer’s mutational profile and subsequent treatment outcomes for patients of different racial and ethnic backgrounds is important to ensure that genomic testing optimally benefits all patient populations. However, many of the studies we reviewed fail to provide information on patients’ racial and ethnic background. When provided, analysis of the type and frequency of gene variants, and the safety and efficacy of ensuing treatments, is not usually stratified by racial and ethnic groups. In most cases, individual studies lack the requisite statistical power for such analyses. Future research is needed to address these evidence gaps.

Finally, numerous stakeholders raised concerns about NGS technology’s potential to exacerbate existing health care disparities in underserved communities. Concerns ranged from lack of insurance coverage to inadequate training or educational opportunities for clinicians and patients. Opportunities exist for future research to elucidate these disparities and to explore ways to broaden dissemination of these new technologies to help benefit all patients with cancer.
Introduction

Genomic testing is a critical component of precision medicine. It has influenced many aspects of patient care, including diagnosis, disease monitoring, and treatment choice. Advances in cancer management, primarily in the form of recently developed targeted drug therapies, depend on companion diagnostic (CDx) tests that identify gene variants to inform the best candidate therapies for a particular patient. The relationship between gene variants and treatment has become so integral in some cases that trials testing new therapies have been organized around a tumor’s genetic profile rather than the specific tissue type where the cancer originated.¹

Next-generation sequencing (NGS) is a well-developed technology that allows for sequencing many genes at once, thereby providing a comprehensive assessment of a cancer’s genetic profile. This technology has been instrumental in driving the diversity and increasing complexity of commercially available genomic tests for cancer profiling. This area was once dominated by serial application of single-gene tests, in which tests searched one gene at a time for critical biomarkers that could help inform a patient’s treatment. NGS-based testing now permits cancer genomic exploration to proceed more rapidly, with hundreds or even thousands of genes potentially sequenced at once. This expansion in throughput, however, has increased the complexity of information obtained from these tests, creating informational burdens on those making clinical decisions and presenting challenges for evidence-based assessment of the technology’s impact on patient care.

Project Purpose and Goals

This project’s purpose was to summarize the current state of clinical evidence supporting use of NGS-based genomic testing for guiding management of patients with cancer. The primary goal was to assess the quantity of evidence evaluating the impact, or potential impact, of NGS-based genomic testing on patient health outcomes, commonly termed clinical utility.²⁻⁴ To accomplish this goal, we conducted evidence mapping, which is a systematic approach to summarizing the evidence underlying a broad topic or field of study.⁵⁻⁶ A major objective of evidence mapping is to delineate areas of heavy research interest, where evidence may be plentiful, from areas where evidence is lacking (termed evidence gaps) and to summarize these findings in a visually convenient format. Identifying knowledge gaps is critical to channeling limited research funding to areas of greatest need and to help engage stakeholders in the health care industry who need evidence to guide decisions.⁶

Because evidence maps provide a summary snapshot of evidence for a particular domain at a given point in time, they must be timely. This is challenging because rapidly developing fields, such as genomic testing, can change very quickly, often within the typical duration required to perform a large-scale systematic review. These constraints place a high demand on defining a project scope and methodology that balances timeliness with comprehensiveness and rigor. Arriving at a suitable balance is an empirical process and subject to iterative development with input from relevant stakeholders.

In this report, we use the terms genomic, genomic testing, and genomic profiling to denote the use of NGS-based tests that simultaneously analyze multiple genes (from several to several hundred). Further, our focus is on genomic profiling of existing cancer (ie, somatic testing). This is distinct from germline testing, which involves analysis of genes conferring risk for hereditary cancer, a topic outside the scope of this report. Similarly, other terms used throughout this report have multiple accepted uses but lack a consensus definition in the field. For our purposes, we will use the term variant to refer to any alteration in
a gene, while reserving the term biomarker for variants with demonstrated associations with treatment responses (either positive responses such as longer survival or adverse events). Furthermore, the terms actionable and clinically actionable pertain to genetic alterations with established, or potential, predictive value for response to therapy.

Current Methodologies for Gene Sequencing

Early Sequencing Technologies

Genes are functional units of the deoxyribonucleic acid (DNA) molecule. Because cancer can be driven by genetic events, including various somatic mutations (changes in DNA sequence occurring after conception that are not inherited), gene sequencing in cancer has multiple applications, including diagnosis, treatment management, and prognosis. Gene sequencing is the process of determining a given gene’s unique sequence of nucleotides, chemical structural units of nucleic acids, such as DNA. As applied to cancer profiling and treatment, gene sequencing is used to identify acquired somatic mutations in tumor tissue or hereditary mutations existing in the germline. Multiple gene sequencing methods exist, and techniques have undergone extensive refinement and improvement over the past 3 decades. Most approaches to nucleic acid sequencing take advantage of DNA’s double-stranded structure, which contains 2 linear chains composed of sequences of 4 different basic molecular building blocks, termed nucleotides. Each nucleotide on a strand pairs, through weak chemical interactions, with the nucleotide in the corresponding position on the other “complementary” strand. Because only certain nucleotides can pair, sequencing technologies can analyze the nucleic acid strand’s sequence by determining the sequence of the complementary strand.

First-generation sequencing technologies (eg, Sanger sequencing) are generally used to sequence individual genes. These techniques employ chemical treatments (eg, enzymes or other chemicals) that break the nucleic acid to generate randomly terminated complementary strands of the molecule. In the laboratory, nucleotides are added to the existing strands through use of DNA extension reactions employing the same enzymes (polymerases) responsible for replicating DNA inside cells. Specially engineered nucleotides (dideoxy nucleotides) that can terminate the replication process are labeled with unique fluorescent tags (one for each type of nucleotide) and added to the reaction, resulting in strands of different lengths, each terminating with a particular labeled nucleotide. The nucleotide sequence is then determined by identifying the particular fluorescent tag on the terminal nucleotide that was incorporated into the different sized single-stranded molecules (the sequence by synthesis approach). To do this, the different fragments must be separated by size, typically using an electrochemical method, termed electrophoresis, that separates molecules based on size and electrical charge.
While the modern and highly refined Sanger sequencing technique is still considered the gold standard for many applications that require sequencing short stretches of nucleic acids with high accuracy, the size-based detection method imposes a substantial bottleneck that limits the number of samples that can be processed simultaneously. Therefore, researchers have sought novel methods for gene sequencing.

**Development of NGS Technology**

Sanger sequencing remained the main technology for sequencing genes until the mid-2000s, when more modern techniques collectively known as NGS, or massively parallel sequencing, began to supersede it. While the Sanger method has only limited potential to sequence multiple genes simultaneously, NGS is massively parallel, making possible the sequencing of hundreds to thousands of genes at a time. This approach permits the search for, and potential discovery of, many different genetic variants at once, yielding a cancer’s *genomic profile*. Different NGS technologies have been developed, including pyrosequencing (generally considered antiquated); Ion Torrent technology (Thermo-Fisher); Illumina Technology (Illumina, Inc), which uses the *bridge amplification* process; and long-molecule sequencing (Pacific Biosciences). While an extended discussion of these complex and diversified methods is beyond the scope of this report, the key practical difference between Sanger sequencing and NGS is the volume of sequencing performed.

**Advances in NGS Technology**

**Liquid Biopsy Tests**

Technological advances that have made high-throughput sequencing possible have quickly affected the commercial genetic testing industry’s growth and product offerings. The relatively recent advent of *liquid biopsy* tests that isolate and sequence DNA from cancerous cells that travel freely in the bloodstream (circulating tumor DNA [ctDNA]) has allowed the sequencing of cancer-related genes from a patient’s blood sample. This technology has permitted repeated, minimally invasive genetic profiling of tumors for guiding ongoing and adaptive treatment regimens.

**Emerging Technologies in Genomic Sequencing**

Targeted NGS gene panels are currently the most frequently used multigene analysis tools in clinical contexts. Targeted panels typically analyze a few to several hundred individual genes at once. NGS can also be used to perform WGS, WES, and RNA sequencing (RNA-seq), which capture a much larger proportion of the genome. WGS analyzes almost all the nucleotides in the genome, including chromosomal DNA and mitochondrial DNA, while WES sequences all protein-coding regions of the genome (ie, the exome). RNA-seq reveals the sequence of RNA transcripts in the tissue of interest. Depending on the RNA preparation method, RNA-seq may assay all cellular RNAs (eg, messenger RNA [mRNA], ribosomal RNA, transfer RNA), or a subset of RNAs (eg, protein-coding mRNAs). In addition to determining the sequence of these RNAs, RNA-seq also provides for analysis of gene expression levels, which can indicate whether genes are aberrantly overexpressed or underexpressed. However, gene expression analysis, while a relatively new and important technique for evaluating cancer, is outside the scope of this report. From a clinical standpoint, these latter techniques, such as WGS, WES, and RNA-seq, can be considered emerging technologies because they are used more often in research and less frequently in the clinical setting, though this will likely change in the coming years.
WES/WGS

While targeted sequencing with multigene panels has been widely adopted in oncology, limitations to this approach exist. Targeted panels assess only a subset of a patient’s genes and therefore may miss potentially relevant genetic variants, particularly rare but potentially actionable genetic variants harbored by cancers. As discovery of novel targeted therapies and cancer-related genetic variants accelerates, targeted sequencing approaches will require regular updating to ensure identification of all potentially actionable genetic variants. Therefore, interest exists in approaches such as WGS and WES that provide more comprehensive genomic analysis. WGS involves sequencing of all the approximately 6 billion nucleotides in the human genome and, therefore, identifies variants in all protein-coding and non–protein coding regions of the genome. Conversely, WES sequences only protein-coding regions of the genome (ie, the exome), which encompasses about 2% of the human genome but contains most disease-causing variants. Therefore, compared with WGS, WES reduces the amount of sequence data generated while still capturing most genetic variants of interest. Historically, the high cost and complexity of WES and WGS have largely limited their use to preclinical studies assessing the genetic architecture of various tumor types. However, with decreasing sequencing costs and increased familiarity with NGS-based methods, investigators have begun to incorporate WES and WGS into clinical settings.

Widespread adoption of WES/WGS into clinical practice faces several technical hurdles. Compared with targeted sequencing panels, WES/WGS typically requires a sample containing genetic material of a higher quantity and quality. Therefore, WES/WGS approaches may require fresh biopsies if genetic material from archived samples (eg, formalin-fixed, paraffin-embedded tissues) is inadequate in quantity or quality. Additionally, WES/WGS may have longer turnaround times compared with targeted sequencing due to increased sequencing complexity and increased bioinformatics burden to identify more variants and determine their potential significance. Finally, WES/WGS are typically performed at reduced sequencing depth (ie, the number of unique reads at each nucleotide) compared with targeted sequencing. This may compromise the detection of subclonal genetic variants (ie, variants not present in every cell of the sample) that could affect treatment choice or ability to detect potential resistance mechanisms.

In addition to technical concerns, increases in identified variants with these techniques can complicate interpretation of test results. Compared with targeted sequencing, WES/WGS will identify more variants with no known clinical utility (ie, variants of unknown, or uncertain, significance). Additionally, WES/WGS may generate incidental or secondary findings related to disease susceptibility, unrelated to the diagnosed cancer. These findings may raise ethical concerns about informing patients and/or family members about these risks. Further, addressing these risks may place an additional treatment or surveillance burden on these individuals. This issue may be particularly important when WES/WGS sequencing of normal (ie, nontumor) tissue is performed in parallel with tumor tissue sequencing to differentiate somatic variants (ie, biomarkers) typically used for guiding treatment choice from germline (hereditary) variants used for assessing disease risk.

Multiomics

Precision medicine has focused on characterizing DNA sequences comprising the tumor genome; however, assessing other aspects of the tumor’s biologic makeup may assist with patient management decisions. DNA carries instructions controlling cellular processes but does not carry out these processes itself. Rather, instructions encoded in DNA must be copied into ribonucleic acid (RNA) through a process called transcription. In turn, the instructions encoded in RNA are used to create proteins through a
process called translation. Therefore, assessing presence and quantity (ie, levels) of specific RNA species and proteins may complement DNA sequence analysis by providing additional information about the activity level of various genes. Because proteins play critical roles in cell metabolism and tissue growth and repair, quantifying their production rate can provide additional important information about the state of disease and potentially inform treatment. For example, high levels of the proteins PD-L1 and HER2 are associated with response to therapy with PD-1/PD-L1 immune checkpoint inhibitors (eg, pembrolizumab [Keytruda], nivolumab [Opdivo]) and anti-HER2 antibodies (eg, trastuzumab [Herceptin]), respectively. DNA may also be modified in ways that does not affect the primary sequence information but does influence the activity of genes. The promise of these adjunct measures to provide additional guidance for treatment has expanded the use of integrated genomic profiling to analyze multiple genome-scale biomarkers (ie, multiomics).21

RNA expression analysis at the whole-genome level (ie, transcriptomics) is a technology frequently used in combination with DNA analysis. In recent years, an NGS-based approach to transcriptome analysis—RNA-seq—has emerged. RNA-seq provides information on differential gene expression, and certain gene expression profiles may allow for diagnosing cancer, assessing prognosis, or predicting treatment response. Unlike older gene expression techniques (eg, microarray analysis), RNA-seq also provides detailed sequence information and, therefore, may identify transcribed genetic variants (ie, variants in the sequence of RNA molecules). In particular, informatics algorithms allow detection of transcribed gene fusions, composite genes formed from 2 previously independent genes. These variants have been identified as common driver events underlying the pathiology of various cancers and are associated with response to treatments (eg, imatinib in chronic myelogenous leukemia with BCR-ABL fusions (breakpoint cluster region protein/tyrosine kinase ABL1), crizotinib in non-small cell lung cancer [NSCLC] with ALK [anaplastic lymphoma kinase] fusions).22

Protein analysis at the whole-genome level (ie, proteomics) is another approach with the potential to complement DNA sequence data. Proteins are the end product of many genes. While RNA expression provides a measure of a gene’s activity, protein expression is regulated after transcription by an array of processes, including translational regulation, posttranslational modification (eg, phosphorylation), and subcellular localization. All these processes may affect protein function and, in turn, cancer biology. Proteomics has lagged behind genomic assessment of DNA and RNA due to the increased complexity of analytic methods for proteins. Therefore, proteomic approaches have been confined mostly to preclinical studies. However, recent advances in proteomic technologies, particularly mass spectroscopy, may allow integration of proteomic data into clinical use. For example, Doll et al recently reported on the use of mass spectroscopy–based proteomic analysis of a case of a rare cancer, urachal carcinoma, that was refractory (ie, nonresponsive) to chemotherapy. Their analysis identified multiple proteins that were upregulated (increased in production), including one with known potential for clinical relevance.

Epigenetic assessment at the genome level (ie, epigenomics) may also be used to complement DNA sequencing data. Epigenomics involves the detection of reversible modifications to DNA (eg, cytosine methylation) or DNA-associated proteins (eg, histone acetylation) that can affect gene function without changing the primary DNA sequence. Epigenetic modification of single genes or groups of genes is associated with various cancer phenotypes and may provide additional biomarkers that could influence treatment.
**Disease Monitoring and Minimal Residual Disease**

While surgical resection or pharmacotherapy can substantially reduce the number of cancer cells, many successfully treated patients will experience disease recurrence. Recurrence is thought to be driven by the persistence of tumor cells following treatment at insufficient levels to be detected by conventional modalities. The high sensitivity of NGS-based assays for detecting tumor DNA has spurred interest in using genomic testing to detect the presence of these cancer cells before macroscopic disease is evident. Detection of this so-called minimal residual disease (MRD) represents a potential prognostic marker for disease recurrence and may allow additional treatment of patients to commence while burden of disease is low.

Hematologic malignancies are particularly amenable to NGS-based MRD testing, owing to the residence of malignant cells in readily accessible locations (ie, bloodstream, bone marrow). Additionally, NGS-based MRD testing in hematologic malignancies has taken advantage of rearrangements in immunoglobulin genes during blood cell maturation. Individual blood cells harbor unique rearrangements of these genes; therefore, a specific rearrangement can be identified and serve as a marker for the presence of the clonal malignancy. Accordingly, an in vitro diagnostic test based on this principle, clonoSEQ®, received US Food and Drug Administration (FDA) clearance (September 2018) for detecting MRD in patients with multiple myeloma and acute lymphoblastic leukemia. Signatera™ is another example of a widely available, commercial ctDNA test for detecting MRD. In contrast, solid tumor cancers are less amenable to MRD monitoring because most cells are localized in small clusters that are below detection limits and, therefore, impossible to biopsy. However, investigators have begun testing an alternative pool of cancer-derived nucleic acids from ctDNA (ie, liquid biopsy tests) to perform MRD testing.

Two general approaches have been taken to detect ctDNA: targeted methods, personalized to the patient’s tumor, and nontargeted methods. Targeted NGS methods typically use a sample of the primary tumor to identify variants of interest using WES, WGS, or a targeted sequencing panel. Once tumor-specific variants are identified, highly sensitive assays can be developed to detect these variants in a patient. In nontargeted methods, WES, WGS, or an NGS panel is used to analyze ctDNA, and informatics approaches are used to detect potential cancer-specific variants. Targeted approaches are generally more sensitive and, therefore, may be better suited to detecting MRD in patients expected to have low levels of ctDNA (eg, patients with nonmetastatic cancers). In contrast, nontargeted approaches require more starting material and are less sensitive. While this renders them unsuitable for detecting initial signs of disease progression, particularly in patients with low levels of ctDNA, the nonbiased assessment of the full genome means these assays may detect novel (de novo) mutations or mutations specific to small subclonal cell populations in the initial tumor.

**Evidence Frameworks Addressing NGS Tests for Patients with Cancer**

NGS technology’s explosive advancement has driven development of increasingly complex and varied tests for risk assessment, diagnosis, and genetic profiling of cancer. Confusion about the purpose and intended patient populations of these different products, uncertainty about their potential to live up to developer claims, interpretation of variants of unknown significance, management of incidental findings (variants not related to investigated disease), and the substantial and rapidly changing costs associated with these tests have generated challenges to multiple stakeholder groups, including clinicians, payers,
Unsurprisingly, proper evaluation of genetic tests has often lagged behind the advancement and availability of marketed products purported to enhance treatment. To respond to this shortcoming and standardize and tailor evidence assessment to genetic test technologies, several evidence assessment frameworks have been developed or applied for assessing the quantity and quality of evidence for genetic tests. These include ACCE (analytic validity; clinical validity; clinical utility; and ethical, legal, and social issues) and, perhaps the most widely cited, EGAPP (evaluation of genomic applications in practice and prevention), an adaptation of the ACCE model. These frameworks have helped define the critical domains of evidence assessment pertaining to genetic tests and set some preliminary criteria defining satisfactory evidence within each domain.

Of these frameworks, the ACCE and EGAPP approaches have defined the 3 widely accepted evidence domains as applied to genetic tests: analytic validity, clinical validity, and clinical utility. Analytic validity defines the assay’s performance in accurately detecting the biomarker of interest (eg, a gene’s specific mutation). Clinical validity assesses the relationship between the biomarker and the disease state (eg, an oncogene regulating cell proliferation) or the clinical purpose (eg, whether the biomarker predicts response to a drug). Clinical utility characterizes the test’s impact on patient management and health outcomes. The scope of evidence addressing these 3 domains is vast, and our literature review, therefore, required a circumscribed scope to be feasible to meet this project’s time frame. Here, our literature review has focused on clinical utility because this domain most directly measures testing’s impact on patient care.

EGAPP specifies critical outcomes for assessing clinical utility, including improved health outcomes, patient management changes, and selection of or adherence to effective treatment regimens. Some outcomes are more direct measures of clinical utility than are others. Improved health outcomes represent an intervention’s desired end goal (be it a test or treatment) and provide direct measures. For cancer treatment, such outcomes include overall survival, progression-free survival, and treatment response. Treatment selection and alterations or the number or percentage of actionable mutations detected (mutations for which evidence indicates enhanced efficacy for some treatments over others), while included among impact measures, do not directly speak to benefits or harms.
Some stakeholders and organizations have argued for relaxing the criteria that define clinical utility outcomes. For the purposes of this report, we adopted liberal inclusion criteria for clinical utility outcomes to provide a comprehensive overview of available literature on this subject. However, throughout the report, we address how directly these various measures relate to actual health outcomes.

Regulatory Framework for Clinical Application of Genetic and Genomic Testing

Centers for Medicare & Medicaid Services Oversight of Laboratory-Developed Tests

Unlike the rigorous standards imposed for drugs, many genetic and genomic tests are not required to have FDA approval or clearance. Most genetic or genomic tests, including NGS-based tests, are laboratory-developed tests (LDTs). FDA broadly defines an LDT as a type of in vitro diagnostic test that is designed, manufactured, and used within a single laboratory.

The Centers for Medicare & Medicaid Services (CMS) oversees laboratories that perform LDTs under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). Importantly, CLIA does not evaluate the performance, accuracy, or reliability of the LDTs themselves. Under CLIA, FDA categorizes LDTs as “waived,” “moderate,” or “high” complexity according to the following criteria:

- Knowledge required to perform the test
- Training and experience needed to perform the test
- Reagents and materials preparation
- Operational steps characteristics
- Calibration, quality control, and proficiency testing materials
- Test system troubleshooting and equipment maintenance
- Amount of interpretation and judgment required

Accordingly, CLIA quality standard requirements for personnel qualifications and responsibilities are more stringent with increasing test complexity.

Traditionally, LDTs were regulated less stringently because they were judged “relatively simple lab tests” with only limited availability. Thus, LDT developers were spared the more rigorous quality and performance standards required of other diagnostic tests. However, technological advances and ensuing expansions in business models and opportunities led to a large expansion in the diversity, technological complexity, and availability of LDTs.

In 2014, FDA drafted guidance for a regulatory framework for oversight of LDTs and in 2015 reported 20 case studies in which LDTs may have resulted in patient harm. Following public commentary on the initial document, FDA issued a discussion paper in 2017 detailing plans for phased-in oversight. Beginning primarily with serious adverse event and malfunction reporting in the first year, the subsequent 3 years were to include progressive premarket review for new or modified LDTs of high-, moderate-, and low-risk tests, respectively. However, FDA indefinitely suspended pursuit of this plan.
FDA Clearance or Approval for NGS-Based Genomic Tests

Developers are permitted to pursue FDA approval or clearance for their genetic and genomic tests, and some have chosen this route. Clearance is granted through the 510(k) process, which applies to tests or devices considered substantially equivalent to those already legally marketed; 510(k) clearances are designated class I, class II, or class III depending on the associated medical risk (genetic and genomic tests carry little risk and, accordingly, are generally categorized class I). Approval requires a more rigorous evaluation of the existing evidence for safety and effectiveness for intended use.

In 2017, FDA approved FoundationOne® CDx, a 324-gene panel that contains multiple companion diagnostic tests. FoundationOne® CDx, like typical NGS panel tests, began as an LDT. FDA does not enforce premarket review and approval of LDTs. However, the test's developer, Foundation Medicine, requested to enter FDA's recently developed (at the time) Breakthrough Devices Program, which provides guidance to the developer for expediting the collection of supportive clinical evidence and the agency's review of the product. In August 2020, Guardant360®, an NGS-based liquid biopsy assay from Guardant Health, received FDA approval through the same program. Seven NGS tests currently have FDA clearance or approval:

- FoundationOne® CDx
- FoundationOne® Liquid CDx
- Foundation Focus™ CDxBRCA
- Guardant360® CDx
- myChoice®
- Oncomine™ Dx
- Praxis™ Extended RAS Panel

CMS Coverage Models

On March 16, 2018, CMS issued a National Coverage Determination (NCD) for NGS testing (updated in January 2020). This policy applies to patients with advanced stages (stages III or IV) of cancer or recurrent, relapsed, refractory, or metastatic cancer. The policy states, "clinical studies show that genetic variations in a patient's cancer can, in concert with clinical factors, predict how each individual responds to specific treatments."

CMS determined NGS as a diagnostic laboratory test to be "reasonable and necessary" and covered nationally when performed by a CLIA-certified laboratory, ordered by a treating physician, and meeting the following requirements:

1. The patient has
   - Been diagnosed with recurrent, relapsed, refractory, metastatic, or advanced stage III or IV cancer
   - Not been previously tested using the same NGS test for the same primary diagnosis of cancer. Repeat testing with the same NGS test is allowed only when the treating physician makes a new primary cancer diagnosis.
   - Decided to seek further cancer treatment (eg, therapeutic chemotherapy)
2. The diagnostic laboratory performing the NGS test must
   - Have FDA approval or clearance as a companion in vitro diagnostic
   - Possess an FDA-approved or -cleared indication for use in that patient’s cancer
   - Present results for management of the patient to the treating physician using a report template to specify treatment options

The NCD was created after a systematic review of studies evaluating NGS-based multigene panels with an emphasis on those reporting on patient mortality, overall survival, and progression-free survival. The NCD will likely evolve along with the evidence base. For example, the current NCD permits only a single use of an NGS test, which was intended to guard against investigational or unnecessary repeat use of testing that does not improve outcomes. However, future revisions may sanction repeat testing for MRD, disease progression monitoring, and other novel purposes. CMS has plans to revisit this topic in future updates to its policy (these topics were discussed with one of our payer stakeholders).

While CMS has determined NGS testing to be reasonable and necessary, numerous local coverage determinations (LCDs) also exist to address NGS testing for specific cancer types or for patients specific criteria or for specific techniques (eg, liquid biopsy). While the aforementioned NCD supersedes individual policies, they may specify additional criteria regarding coverage policy for specific tests not covered by the NCD or conditions criteria specific to their topic.

Palmetto GBA developed the Molecular Diagnostics Services (MolDX®) Program in 2011 to identify and establish coverage and reimbursement for molecular tests. This program establishes clinical utility expectations, completes technical assessments of published data to determine clinical utility and coverage, and establishes reimbursement, among several important functions. LCDs are generally established by the MolDX® Program before incorporation into other Medicare Administrative Contractor (MAC) policies. Appendix A displays LCDs from a variety of MACs and summarizes coverage indications, limitations, and medical necessity for NGS-based multigene panel tests for cancer treatment. In general, these policies state explicit patient indications (eg, cancer type) and apply to patients at diagnosis, in the absence of response to treatment, at relapse, or at disease progression. While some LCDs refer to an exemplar test, they will usually cover similar tests if supporting evidence and intended use are within the policy’s scope, according to a comprehensive technical assessment. (Note: these policies can change frequently, so the list we provide may be incomplete or modified by the time of this report’s publication.)

NGS in Clinical Practice

Widespread adoption of a particular technology may be assessed through an examination of clinical practice guidelines, which are published documents intended to help clinicians manage patients according to the best, most timely evidence and in a uniform and standard manner. Notably, several clinical practice guidelines state that NGS testing may be used to detect established biomarkers that can help select treatment with FDA-approved therapies or match patients with clinical trials testing new therapies. These guidelines vary in breadth and scope. Some address use of NGS technology, in general, while others discuss the evidence for or against use in specific indications. Importantly, multigene panel testing may not be the best choice for all patients, in all situations. For example, when variants of interest and corresponding therapies are very limited in number, single-gene testing may provide a better option. Cancer type and patient history are factors to consider when selecting the best testing strategy.
Not surprisingly, indications suggest that physician utilization and attitudes regarding NGS testing align with the increased attention to this technology in guidelines. One survey from 2018 found that approximately 75% of the more than 1200 oncologists queried reported use of NGS tests to guide treatment decisions. However, with increased uptake, numerous challenges have surfaced. Evidence indicates that many oncologists lack confidence in their ability to interpret, discuss, or use NGS test findings. Other challenges relate to reimbursement for testing and access to drugs on or off clinical trials. Further, concern surrounds interpretation of results from NGS testing, once administered. Test results can provide a large volume of technically complex information, and the continued expansion in the size of multigene panels over the past several years has intensified this concern. Additionally, testing performed for cancer management purposes can yield findings important in other contexts, and this may require ancillary clinical support. For instance, one study noted that oncologists valued genetic counselor involvement when incidental germline findings were identified. Given these considerations, it is perhaps unsurprising that the aforementioned survey study reported that oncologists who were younger than 50, held a faculty appointment, received genomics training, and saw more than 50 unique patients per month, or who had access to a molecular tumor board, were more likely to use NGS tests.

Guiding Questions for This Review

Here we provide a brief overview of the major sections of this report and a prelude to our evidence map analyses. In structuring our review’s scope, we considered the following guiding questions, provided by PCORI:

1. What genetic panel tests are available in guiding cancer treatment/management? Do these improve patient outcomes?
   a. FDA-approved applications, including use as a companion diagnostic
   b. Applications in practice not subject to approval (eg, completed through a CLIA-certified laboratory)
2. What evidence is available evaluating benefits (eg, leading to a highly effective targeted therapy) and/or harms (eg, secondary findings, choice of therapy without evidence) of the use of these applications?
3. What applications are currently being evaluated with new research, or are otherwise being developed, that may be expected to be adopted into clinical care in the next 5 years?
4. What health conditions (ie, cancer types), health care interventions, or other characteristics of patients, care, and systems are being addressed by genetic sequencing?
5. For the current and future applications evaluated above, where does the evidence fit within the chosen framework of diagnostic and prognostic testing?
6. Based on the review, what evidence gaps could future research solve?

In addition to these guiding questions, the ECRI team and PCORI staff conducted calls with individuals representing various stakeholder groups with interest in this topic, including clinical experts (medical oncologists), representatives from government and commercial payer organizations, patients, and representatives from patient advocacy groups. During these calls, additional topics and questions of interest included (1) the availability and dissemination of NGS-based genomic testing across different underserved populations and geographic regions; (2) the reporting and stratification of data on NGS testing for patients of different racial and ethnic backgrounds; (3) the challenges for patients, clinicians, and payers with use of this technology; and (4) the personal experiences of patients with cancer regarding
genomic testing’s impact on their care. Appendix B provides further details on these stakeholder interviews and the guiding questions the ECRI team asked to frame the discussions.

Report Organization

After briefly reviewing our methodology, below, our presentation of results begins with a broad overview of the evidence base in terms of cancer types, study design (eg, single-arm or comparative, prospective or retrospective), and the key outcomes reported. We address the major trends in the types of evidence presented and note where evidence is most lacking, particularly as it affects the evaluation of clinical utility of NGS genomic tests. We then describe the evidence comparing outcomes of patients treated with genomic-guided or genomic-matched therapy vs comparator patients treated without guided therapy or with single-gene testing or smaller panels. This evidence bears most directly on evaluation of NGS-based testing’s clinical utility. We then present evidence map summaries for the single-group observational studies. Though we cannot use these studies to evaluate rigorously the impact of NGS tests, they composed most of the literature base and provide a profile of the cancer types examined, the specific tests most studied, and the distribution of outcomes reported.

The concordance between mutations identified by cell-free DNA or ctDNA (ie, liquid biopsy) and tissue-based NGS tests is a major concern because of the newer and more technically demanding liquid biopsy assays that isolate ctDNA from blood samples before performing gene sequencing. Such studies provide a convenient, if not thoroughly rigorous, measure of the clinical validity of these recently developed tests. We also briefly discuss a small class of studies that report associations between gene mutations or other biomarkers from NGS testing and cancer types or outcomes with use of targeted therapies. Most notable among these are the studies of the recently FDA-approved myChoice® CDx test.

Also included is a narrative review of studies using key emerging technologies. We describe the types of techniques in use and purposes for their use and project the impact of these technologies on cancer treatment over the next 5 to 10 years. Finally, we provide a brief summary of ongoing clinical trials relevant to this topic. We conclude by discussing our findings in a broader context, with respect to evidence domain frameworks developed for genetic testing. We then highlight some of the major evidence gaps in the literature, along with reasons the gaps might exist, and suggest some paths forward for addressing them, particularly regarding evaluations of clinical utility.
Methods

Here we provide a brief overview of our methodology and inclusion criteria. Additional details on the literature search strategy and literature review procedure appear in Appendices C and D, respectively. Our literature flow diagram, outlining the results of our literature screening process, appears in Appendix E.

Project Scope

We targeted our literature review to studies using NGS-based panel tests assessing the use of somatic, or biomarker, testing in patients with cancer. Such testing concerns analysis of gene variants occurring after conception and not those present within the germline (ie, indicating predisposition for hereditary cancer). Such studies have direct relevance to cancer treatment. We accepted studies of any design that reported on one or more outcomes of interest, including direct measures of clinical utility (eg, overall survival, progression-free survival), decisions or changes regarding patient management based on test results, or reports of the number or percentage of actionable mutations. Other accepted surrogate outcomes for clinical utility are detailed below. We provide additional detail on literature screening methods in Appendix D.

We did not include studies of analytic validity (the accuracy and reproducibility of laboratory assay performance) or clinical validity studies focused on demonstrating relationships between genetic variants assayed by the test and the cancer type or disease state. We also excluded studies focused on single-gene tests or tests employing techniques other than NGS-based genomic sequencing (eg, fluorescent in situ hybridization [FISH], gene expression analysis), as well as studies of genomic testing strictly intended for disease prognosis (without evaluating treatment options).

We also limited our final selection to studies of the most widely available, commercial NGS panel tests. Therefore, we excluded from our analysis studies using research use–only tests or tests not widely accessible to all patients (eg, tests offered only to patients within a specific health system or academic facility). Regarding the latter criterion, we had concerns about the generalizability of results from system-specific tests, both in terms of the patient populations examined and test utilization patterns (eg, clinicians may be more apt to use in-house tests and act on their results). Regarding research use–only tests, we made exceptions for studies pertaining to emerging technologies, such as WES or WGS, because these technologies are primarily used in research and commercial tests and are not yet widely available. We limited our analysis to studies employing tests commercially available in North America and from CLIA-certified laboratories.

Specific Inclusion Criteria

We considered studies with the following characteristics:

- Studies of any design that present data and/or patient outcomes on the use of widely available commercial NGS-based genomic tests
- Studies published in English
- Studies published January 1, 2010, through August 28, 2020

Inclusion criteria were guided by a framework specifying the patient population, intervention, comparisons of interest, and outcomes of interest (PICO). Extensive discussions with PCORI and
subsequent interviews with clinical experts and payer representatives informed the framework’s specifications. For this project, the PICO framework was as follows:

- **Patient population:** Patients given a cancer diagnosis
- **Interventions:** Cancer genomic profiling with an NGS-based test
- **Comparisons:** Patients managed without NGS testing or with a comparator test (single-gene or other NGS panel tests)
- **Outcomes:**
  - Reports on clinically actionable mutations (ie, mutations with potential to guide treatment)
  - Management decisions or changes in treatment
  - Patient health outcomes (including overall survival, progression-free survival, treatment response/stable disease, time to treatment failure, and enrollment in clinical trials)
  - Adverse events or toxicity (due to treatments)
  - Surrogate outcomes of clinical utility (as specified below)

Through discussions with PCORI, we agreed on additional studies reporting surrogate outcomes with potential relevance to clinical utility. These outcomes included the following:

- Physician or patient perspectives of test results (eg, clinician or patient perspectives about genomic testing or confidence in treatments prescribed or received)
- Concordance data between detection of genetic variants in liquid biopsy– and tumor biopsy–based tests
- Diagnostic accuracy measures of tests intended to detect disease progression or recurrence (as these data could inform therapies for some patients)
- Projected impacts of genomic testing on patient care from cost-effectiveness studies (including real-world data)
We excluded from our review studies with the following characteristics:

- Focused on evaluating analytic validity
- Focused on evaluating non–NGS based tests (unless used as a comparator)
- Genomic panel tests limited to patient populations at specific institutions or health systems and not widely available
- Tests for evaluating prognosis and lacking objective performance measures
- Tests evaluating cancer risk (ie, germline testing)
- Experimental or proof-of-concept studies*
- Studies using research use–only tests*
- Studies of development or testing of animal models
- Individual case reports on one or a few patients (n < 10)
- Studies published only as conference abstracts
- Narrative reviews, editorials, letters, or news reports

*Research use–only tests and proof-of-concept studies were permitted for studies of emerging technologies.

**Literature Search**

An experienced medical research librarian performed all searches for this project. Our comprehensive search protocol included searching EMBASE.com (EMBASE and MEDLINE combined) for studies published between 2010 and 2020 and added to the database on or before August 28, 2020. Our date range was limited back to the year 2010 because most NGS-based tests for cancer profiling came to market in later years. Preliminary searches indicated a sharp decline in the number of potential studies of interest before that year. We present the strategies in EMBASE.com syntax (using EMTREE) in Appendix C.

We also searched ClinicalTrials.gov, the PCORI website (Research and Results and Topics areas for relevant publications), ECRI’s Genetic Test Assessment database, the Google search engine, Google Scholar, our in-house leads database for the PCORI Healthcare Horizon Scanning System, selected third-party payer websites, CMS’s website, and FDA’s website. We detail our search strategies and rationale for selecting each resource in Appendix C.
Stakeholder Interviews

ECRI conducted calls with a range of stakeholders, including health care providers, representatives from government and private payer organizations, patients, and representatives from patient advocacy groups. ECRI conducted 12 calls with 10 stakeholders, including 2 with each clinical expert (clinical oncologists). These interviews were intended to garner input into the project’s scope and content and to solicit patient perspectives on NGS technology’s use in their care.

Conversations with clinical experts focused on report scope, outcome measures, the importance of measuring clinical utility, and the technology’s impact on patient care. One of the 2 clinicians had experience in health care disparities, so we conducted an additional call with this stakeholder to discuss this topic. With payer organization representatives we addressed the subject matter and project scope, payment models for NGS-based tests, assessment of evidence for policy decisions, and factors that influence coverage policies on tests (such as guideline recommendations or FDA approvals). Talks with representatives of patient advocacy groups focused on the technology’s importance for patients; the knowledge about, experience with, and understanding of the technology on the part of clinicians and patients; and health care disparities and socioeconomic impacts on access to genomic testing and subsequent care. We also discussed contributions of the respective organizations to patient education and facilitation of access to testing and care options.

Appendix B provides a list of guiding questions for discussions with each stakeholder group. We address key issues raised by stakeholders in the context of our findings and discuss their insights throughout the report, particularly in the Discussion section.

We interviewed the following key informants (patients’ names are excluded for confidentiality):

Clinicians

- Edith P. Mitchell, MD, MACP, FCPP, FRCP (London); clinical professor of Medicine and Medical Oncology, director, Center to Eliminate Cancer Disparities, associate director, Diversity Affairs, Sidney Kimmel Cancer Center at Jefferson Health
- Mark Robson, MD; attending physician, Memorial Sloan Kettering Cancer Center

Insurers

- Eugean Jiwanmall, MPH, MBA; senior research analyst, Medical Policy & Technology Evaluation, Medical & Claim Payment Policy—Medical Affairs, Facilitated Health Networks, Independence Blue Cross
- Bryan Loy, MD, MBA; physician lead—Oncology, Laboratory and Personalized Medicine, Humana
- Jaime L. Natoli, MPH, MS, CGC; senior consultant, Evidence-Based Medicine Services Unit; Kaiser Permanente, Southern California Permanente Medical Group
Public policymaker

- Carl Li, MD, MPH; medical officer, CMS

Patient advocates

- Kristen Santiago; senior director, Public Policy Initiatives, LUNGevity
- Lisa Schlager; vice president, Public Policy, FORCE (Facing Our Risk of Cancer Empowered)
- Dicey Jackson Scroggins, MA; director of Global Outreach & Engagement, International Gynecologic Cancer Society
Results

1. Overview of Evidence Base

General Designs of Included Studies

We included 321 studies exploring the use of widely available commercial NGS tests for treating patients with cancer (see Appendix F for a list of tests used in the studies included in our evidence map analyses). Figure 1 displays the number of studies with the most common designs. The overwhelming majority of the evidence base consists of single-arm observational studies. All participants in these studies underwent NGS-based testing, and results were reported, sometimes along with other outcomes (eg, treatment decisions or health outcomes), after a specified, often variable, follow-up period.

Figure 1. Included Studies Stratified by Design and Patient Registration

Abbreviations: NR comparative, nonrandomized comparative studies (cohort or post hoc comparisons); RCT, randomized controlled trial; Single-arm obs, single-arm observational; SR, systematic review.

Single-arm observational studies were the most prominent design represented in our evidence base. Note that our searches identified no randomized controlled trials evaluating NGS testing’s impact. Associational studies mostly undertook post hoc explorations of detected biomarker variants and their relation to patient outcomes (see Section 6 of Results section on Association Studies). By convention, systematic reviews are labeled as retrospective. Note: this graph does not tally all included studies. Figure 2 presents numbers on cost-effectiveness and questionnaire studies not presented here.
An intervention’s effectiveness is typically measured through high-quality randomized controlled trials (RCTs). This consensus is reflected in existing frameworks for evidence assessment on genetic tests.\(^3\) Despite this accepted standard, we did not identify any RCTs designed to evaluate the clinical impact (ie, clinical utility) of commercial NGS-based genomic testing. A few studies were RCTs by design, but the randomization did not pertain to studying the results or impact of NGS tests. Nonrandomized comparison studies were the next largest category. This group included comparative cohort studies or studies that provided comparative data for evaluating NGS testing’s impact. For instance, single-arm studies that included post hoc comparisons of interest (eg, comparing patients managed with or without genomic testing) were included in this category. Overall, most studies in these categories were retrospective, reflecting methodologically less-rigorous evidence. Studies with retrospective patient registration suffer from higher risk of bias and, accordingly, are considered lower-quality designs.

Associational studies were a separate category consisting of largely exploratory studies of genomic test results and patient outcomes or clinical management choices. Most of these studies were retrospective in nature, though some were controlled trials investigating drug therapies but which performed post hoc analyses to identify biomarker-health outcome relationships. Part 6 of this section provides more detail on these studies.

**Reported Outcomes and Subject Matter Areas of Included Studies**

The selected studies reported on one of several outcomes or general topic areas of interest: (1) patient health outcomes,\(^{54-155}\) (2) patient management decisions/changes,\(^{54,55,64-66,75,78,81,83,86,88,90,91,93-95,100-104,106,107,110-112,114,116,117,119,120,122-124,128,130,132,134,135,137,140,142,143,145,147,149-153,155-178}\) (3) clinically actionable mutations,\(^{54,55,64,66,74-77,156,179-191}\) (4) concordance of liquid biopsy and tissue biopsy testing,\(^{55,83,91,107,126,128,140,145,161,162,192-223}\) (5) tumor mutational burden (TMB),\(^{75,79,80,82,84,85,89,95,97,105,118,121,135,138,163,176,186,224-232}\) (6) cost-effectiveness and modeling data (examined for NGS testing’s estimated impact on clinical outcomes),\(^{133,171,233-242}\) (7) patient/physician interviews or questionnaires,\(^{50-53,111,243-255}\) and (8) studies of emerging technologies (eg, WES, use of MRD).\(^{15-18,26,256-326}\) Figure 2 displays the number of studies identified reporting on each of these 8 categories of interest. Modeled estimates of effectiveness from studies of cost effectiveness and patient/physician attitudes were considered for surrogate outcomes of clinical utility, and studies of emerging technology were of interest for projecting future trends for NGS technology. These studies were variable in their outcome measures, so we included them here to display additional topics of interest.
Figure 2. Number of Studies by Reported Outcomes of Interest and Topic Areas

Abbreviations: CE data, cost-effectiveness data (examined only for models of NGS testing impact); LB vs tissue, liquid biopsy vs tissue biopsy concordance data; Phys/pt quest., questionnaire studies of physician and patient attitudes/perspectives toward genomic testing; TMB, tumor mutational burden.

Bar graph of the number of studies reporting on outcomes or broader topic areas of interest. Some studies fit into more than one category, so the total count across all bars is greater than the total study count. Studies reporting clinically actionable mutations were most common, followed by studies reporting patient health outcomes and patient management decisions. Questionnaire studies included surveys of patients and/or physicians and reported attitudes, experiences, and preferences regarding genomic sequencing. Emerging technology studies included tests using WES and WGS or studies reporting on minimal residual disease or disease progression monitoring. Cost-effectiveness studies were included only for examining estimated impacts of NGS testing on clinical outcomes.

Figure 3 summarizes the overall evidence base by the most frequently used commercial tests and study design (prospective or retrospective). Most of the studies in the entire evidence base (66%) were retrospective in nature (Figure 3A). Of those studies using a single NGS test, FoundationOne® (original and CDx tissue-based tests offered by Foundation Medicine) had the most published evidence (106 studies), followed by Guardant360® (Guardant Health; 57 studies) and studies using multiple tests (44 studies; Figure 3B).
Figure 3. Most Frequently Studied Commercial NGS Tests

A: Pie chart showing most studies were retrospective. Note that studies with indeterminate or unspecified enrollment were excluded from this figure.

B: Abbreviations: CDx, companion diagnostic; Dx, diagnostic; F1, FoundationOne®.

Most NGS studies were conducted with FoundationOne® solid tumor tests or Guardant360® liquid biopsy assay. Multiple tests category used 2 or more tests on patients.

Cancer Types Represented in Our Evidence Base

We stratified studies according to 11 of the most common cancers, as compiled by the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) Program, as follows: breast, lung, prostate, colorectal, melanoma, kidney and renal pelvis, bladder, melanoma, kidney and renal pelvis, prostate.

We also included studies on patients with one of several different cancer types (“multiple”), or “other” cancers, which were not among the 11 most common cancers. Other cancers include all cancer types not listed in the figures below (eg, gastric, ovarian); see Appendix G for a complete list. Figure 4 displays the number of studies stratified by cancer type and participant registration (prospective or retrospective). Among commonly studied individual cancers, most studies reported on lung (61 studies) or breast (24 studies) cancer. However, more studies (111 studies) reported on a mix of patients with different cancer types compared with studies that focused on any one cancer.
Figure 4. Included Studies Stratified by Cancer Type and Patient Registration

![Bar chart showing the number of studies and patient registration by cancer type.](chart)

**Abbreviation:** RP, renal pelvis.

The total number of studies and patient registration (prospective or retrospective) by cancer type. Studies reporting on patients with one of several different cancer types were most common. Studies reporting on "other" cancer types and lung cancer are the next most common.

Simply counting the number of studies does not accurately quantify the data on each cancer type. For example, even though 88 studies reported on "other" cancer types, these studies were generally small (ie, included fewer patients) because the conditions were of lower prevalence. Because of this, we also wanted to represent the number of participants across studies of cancer types. Figure 5 is a bubble plot showing the number of studies for each condition (same as Figure 4) with information on median size of the studies (in number of participants). As expected, we found no obvious correlation between the number of studies and median study size. For most cancer types, the median study size was about 75 to 100 patients. Unsurprisingly, studies that included multiple cancer types were the largest, reflecting easier patient recruitment. Studies of uterine cancer had the smallest median size, with fewer than 25 participants.
Analysis of Reported Outcomes by Cancer Type

We generated heat maps to display the number of studies stratified by cancer type and outcome reported and the cumulative number of patients included in these studies stratified by cancer type and outcome reported. Figure 6 and Figure 7 display the findings for all studies. The evidence base largely consists of studies involving patients with lung cancer or “other” cancers, or studies including patients with one of several different cancer types (“multiple”). When considering the total number of patients included in studies in conjunction with the number of publications, it becomes apparent that a relative paucity of data exists for most outcomes of interest. Studies reporting clinically actionable mutations, alone, provide no evidence for NGS testing’s impact on health outcomes, and few data exist on patient management changes or patient health outcomes for most cancers. A clear need exists for additional evidence and/or an alternative approach to evaluating the utility of genomic sequencing tests. Because a significant portion of the published evidence base focuses on FoundationOne® (33.0%) and Guardant360® (17.8%), we performed a more thorough data analysis on these tests (see Appendix H).
Figure 6. Study Count by Cancer Type and Reported Outcomes for All Studies

<table>
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<tr>
<th>Health outcomes</th>
<th>Breast</th>
<th>Lung</th>
<th>Prostate</th>
<th>CRC</th>
<th>Melanoma</th>
<th>Bladder</th>
<th>Kidney and RP</th>
<th>Uterine</th>
<th>Leukemia</th>
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<th>Thyroid</th>
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Abbreviations: CRC, colorectal cancer; RP, renal pelvis. This heat map displays the number of studies on each cancer type reporting on each of 3 outcomes (see far left panel). Darker shades represent higher numbers of published studies reporting data for each outcome for a given cancer. For “Multiple,” patient inclusion was not limited to a specific cancer type. For “Other,” studies reported on a cancer type not listed in figure (see Appendix G for full list).

Figure 7. Number of Patients Stratified by Cancer Type and Outcome Reported for All Tests

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<th>Prostate</th>
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Abbreviations: CRC, colorectal cancer; RP, renal pelvis. This heat map displays the number of individuals with a diagnosis of each cancer type with reported outcomes in studies. Darker shades represent higher numbers of patients included in studies. For “Multiple,” patient inclusion was not limited to a specific cancer type. For “Other,” studies reported on a cancer type not listed in figure (see Appendix G for full list).
2. Comparative Data for Evaluating Clinical Utility

Overview of Evidence

We identified 33 studies on NGS panel tests that addressed a variety of comparisons, but we focused our analysis on 18 studies comparing (1) impact of genomic-targeted treatment to nontargeted treatment on patient outcomes,58,84,90,102,107,124,125,129,136,148-150,171,293 (2) large NGS panels vs single-gene or smaller NGS panels,94,349 or (3) different large NGS panel tests.346

For the 15 publications comparing NGS-guided to unguided treatment, or matched to unmatched therapy,58,84,90,102,107,111,124,125,129,136,148-150,171,293 we looked for and accepted studies that examined the health outcomes of patients with cancer and addressed 1 of 2 comparisons: (1) management with or without NGS-based genomic testing and (2) treatment with or without matched therapies informed by NGS-based genomic testing. Note that the first comparison could include analysis of patients who underwent NGS testing compared with those who did not or comparisons of patients who were all tested but with only a subset being managed with NGS test results. In the matched vs unmatched therapy comparison, generally both groups have undergone testing, with the potential for discovering actionable mutations, but only one group receives matched therapy based on testing results.

Comparative studies reviewed here excluded any studies dealing with cost effectiveness, physician/patient perspectives (questionnaire studies), or emerging technologies. We also excluded studies (1) that used NGS in conjunction with other assays (eg, FISH, immunohistochemistry) with no clear way to delineate each test’s impact and (2) that were lacking a comparator of interest (eg, patients on chemotherapy only). Our searches retrieved 15 additional comparative studies, but we excluded them from analysis because they were not crucial to directly assessing NGS-based testing’s overall impact on patient treatment. These studies focus primarily on identification of biomarkers (without subsequent impacts on treatment) and their relation to treatment outcomes or patient management. Some of the topics include reports of mutational or other variant profiles in different cancer types86,232 or subtypes of cancer,87,93,110,189,352 early- vs late-stage disease,64,329 documenting associations between deleterious mutations and treatment efficacy,68 relations between microsatellite instability and tumor mutation burden with targeted NGS and WES,95 comparing use of different sequencing strategies,340 disease monitoring,64,141 and impact on clinical trial participation in different clinical settings.167

The evidence map analysis that follows pertains to studies comparing genomic-guided to unguided treatment. We then provide brief narrative summaries of studies of large NGS panels vs single or smaller panels and one study comparing different large-scale NGS panel tests. Finally, because of the paucity of comparative data of most interest (15 studies of the 321 total included; 4.7%), in concluding this section, we briefly summarize several additional studies that did not meet our inclusion criteria but did use NGS-based testing and provide additional observations relevant to our discussion.

Guided vs Unguided Management: General Remarks on Study Design and Quality

We found no high-quality studies, such as RCTs or high-quality nonrandomized studies testing the impact of commercial NGS panel tests. Most studies (12 of 15; 80%) are retrospective in design, and some are single-arm studies with post hoc comparisons. The study by Radovich et al 2016125 is unique, being a prospective cohort study designed specifically to compare patients with solid tumor cancers managed with and without NGS testing. This study used the PCDx™ test (Paradigm Diagnostics). The other 2
prospective studies tested patients with the FoundationOne® test for solid tissue. The 3 prospective studies are also of moderate size, enrolling 101, 339, and 640 patients, respectively. The retrospective studies are generally of moderate size, most ranging from 27 to about 400 patients. The study by Singal et al184 is unique in having more than 4000 patients. Most studies (10 of 15; 67%) had one or more authors with financial interests or consulting arrangements with, or who received research support from, the test developer.84,90,102,124,136,148,149,171,293 Each included study’s basic description is included in the first 7 columns of Table 1. Additional details on these studies are available in Appendix I.

Summary of Findings

In Table 1, we classify each study according to the cancer type investigated and the comparison specified by the authors. The exception is the study by Moore et al150 which we included in the guided vs unguided grouping because genetic testing revealed no actionable mutations for some patients. Also notable, the study by Bryce et al111 compared patients receiving genomic-guided therapy vs patients who chose to stay on standard of care treatment. Other reasons for not using genomic-guided therapy included inaccessibility to the treatment recommendation and physician choice not to pursue genomic-guided treatment. Therefore, aside from purely methodologic concerns regarding nonrandomized studies, characteristics of comparison groups were variable and sometimes suffered from considerable risk of bias. For instance, choosing not to pursue guided therapy may indicate positive outcomes for standard of care treatment, or uncertainty regarding impact of guided therapy for the particular patient group. For these reasons, we consider this evidence base to be of low quality. (See Discussion section of Radovich et al125 for comments on bias concerns for their study, which have general applicability to this evidence.)

The list of individual studies, arranged by cancer type studied, appears at the left of Table 1. Subsequent columns list basic information about the study and outcomes. Figure 8 displays the tests used in studies of prospective and retrospective design. Thirteen of 15 of the reviewed studies with comparative data reported on use of a single commercial assay. Most studies reported on exclusive use of the standard FoundationOne® test for use with solid tumor tissue.58,84,102,111,124,129,148,149,293 Guardant360® (gray wedge in Figure 8) was the second most frequently used test (3 studies90,107,136). The study by Moore et al150 used multiple commercial assays, including FoundationOne®, GPS Cancer™, Guardant360®, and Tempus. The study by Reitsma et al171 reported on use of both the FoundationOne® (solid tumor test) and FoundationOne® Heme (test for hematologic malignancies). One study by Radovich et al125 used the PCDx™ test.
Figure 8. Number of Studies by Tests Used and Study Design

Bars display the number of studies using each commercial test for prospective and retrospective designs. Most studies used the FoundationOne® test (light blue panels) or the Guardant360® liquid biopsy test (gray panel). Most studies (12 of 15; 80%) were retrospective in design. Foundation One + Heme used both solid tumor and hematologic tests. Multiple tests used more than one NGS test (see narrative summary for details).
Figure 9 displays a heat map of the different cancer types and the NGS tests used to profile them. Most studies reported on patients with multiple solid tumor\textsuperscript{58,102,124,125,129} (5 studies) or solid tumor and hematologic cancers\textsuperscript{111,129,149} (3 studies). Two studies reported on patients with non–small cell lung cancer\textsuperscript{84,107} and one study each reported on biliary cancer\textsuperscript{148}, colorectal cancer\textsuperscript{90}, pancreatic cancers\textsuperscript{293}, cancers of the head and neck\textsuperscript{136}, and salivary gland carcinoma\textsuperscript{150}.

**Figure 9. Heat Map of Studies by Cancer Type and Test**

<table>
<thead>
<tr>
<th></th>
<th>ST</th>
<th>ST+H</th>
<th>H/N</th>
<th>Biliary</th>
<th>CRC</th>
<th>NSCLC</th>
<th>Pancreatic</th>
<th>SGC</th>
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</thead>
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<tr>
<td>FoundationOne</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>F1+F1 Heme</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PCDx</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>Multiple tests</td>
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<td>0</td>
<td>0</td>
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<td>1</td>
</tr>
</tbody>
</table>

Abbreviations: CRC, colorectal cancer; F1+F1 Heme, FoundationOne\textsuperscript{®} and FoundationOne\textsuperscript{®} Heme tests; H/N, head and neck; NSCLC, non–small cell lung cancer; PCDx, Paradigm PCDx\textsuperscript{™} test; SGC, salivary gland carcinoma; ST, solid tumor; ST+H, solid tumor and hematologic.

Cells display the number of studies investigating each cancer type (top row) and the test used (left column). Darker shades indicate more studies. Most studies (8 of 15) investigated patients with multiple solid tumor or hematologic cancers. Most studies (9 of 15) used the FoundationOne\textsuperscript{®} test.

Note that in Table 1, for some studies, the number of patients of interest is fewer than the total enrollment. Actionable mutations were the most frequently reported outcome (14 of 15 studies; 93%), while 10 studies (67%) reported on overall survival and 9 (60%) on progression-free survival. Fourteen studies (93%) reported on or mentioned management decisions resulting from testing. Twelve studies (80%) also reported on assignment of targeted therapies; 5 of these reported both on-label and off-label use while the other 4 studies did not specify on-label or off-label use. Only 2 studies reported adverse events resulting from therapies (not the tests), with one\textsuperscript{293} citing serious toxicity. Events occurred in 8 patients who were excluded from outcomes analysis. Finally, two-thirds of the studies (10 of 15) had one or more authors with affiliations or financial ties to the NGS test developer. These studies are identified in Table 1’s far right column. Additional data summaries for each study in Table 1, including actionable mutations detected and targeted therapies assigned (when available), are provided in Appendix I.
Table 1. Outcomes and Reported Data for Comparative Studies Assessing Impact of NGS Testing

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Study</th>
<th>Test</th>
<th>Design/comparison</th>
<th>N patients</th>
<th>Cancer stage</th>
<th>Ethnicity reported</th>
<th>OS</th>
<th>PFS</th>
<th>Response/stable disease</th>
<th>Time treatment failure</th>
<th>Actionable mutations</th>
<th>Management decisions</th>
<th>Targeted therapies</th>
<th>Clinical trials</th>
<th>AE/toxicity</th>
<th>Affiliation</th>
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</thead>
<tbody>
<tr>
<td>BIL</td>
<td>Javle et al 2016</td>
<td>F1</td>
<td>R M/uM</td>
<td>321</td>
<td>mA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CRC</td>
<td>Kato et al 2019</td>
<td>GRD</td>
<td>R M/uM</td>
<td>94</td>
<td>mA</td>
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<td></td>
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<tr>
<td>H/N</td>
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<td>GRD</td>
<td>R M/uM</td>
<td>60</td>
<td>A</td>
<td>n</td>
<td></td>
<td></td>
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<td>NSCLC</td>
<td>Singal et al 2019</td>
<td>F1</td>
<td>R M/uM</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Schwaederle et al 2017</td>
<td>GRD</td>
<td>R M/uM</td>
<td>88</td>
<td>A</td>
<td>±</td>
<td></td>
<td></td>
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<tr>
<td>PAN</td>
<td>Pishvaian et al 2018</td>
<td>F1</td>
<td>P M/uM</td>
<td>640</td>
<td>A</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td>±</td>
<td></td>
<td>±</td>
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<tr>
<td>SGC</td>
<td>Moore et al 2019</td>
<td>Mult</td>
<td>R G/uG</td>
<td>27</td>
<td>A</td>
<td>i n</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>ST</td>
<td>Sadaps et al 2018</td>
<td>F1</td>
<td>R G/uG</td>
<td>313*</td>
<td>NR</td>
<td>±</td>
<td></td>
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<td></td>
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<td></td>
<td>Grenader et al 2016</td>
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<td>R G/uG</td>
<td>30</td>
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<td></td>
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<td>Radovich et al 2016</td>
<td>PCDx</td>
<td>P G/uG</td>
<td>101</td>
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<td>±</td>
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<tr>
<td></td>
<td>Schwaederle et al 2016</td>
<td>F1</td>
<td>R M/uM</td>
<td>347</td>
<td>A</td>
<td>±</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Schwaederle et al 2015</td>
<td>F1</td>
<td>R M/uM</td>
<td>392</td>
<td>Mult</td>
<td>±</td>
<td></td>
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<tr>
<td>ST+H</td>
<td>Reitsma et al 2019</td>
<td>F1+</td>
<td>R M/uM</td>
<td>95</td>
<td>Mult</td>
<td>±</td>
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<tr>
<td></td>
<td>Bryce et al 2017</td>
<td>F1</td>
<td>R G/SC</td>
<td>141</td>
<td>NR</td>
<td></td>
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<td>±</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Wheler et al 2016</td>
<td>F1</td>
<td>P M/uM</td>
<td>339*</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>±</td>
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</tbody>
</table>
Table Legend

Cancer type: BIL, biliary; CRC, colorectal cancer; H/N, head and neck; NSCLC, non–small cell lung cancer; PAN, pancreatic; SGC, salivary gland carcinoma; ST, solid tumor; ST+H, solid tumor and hematological.

Colors: Gray panels, data reported (no statistical comparison or statistical insignificance); white panel, data not reported; dark blue panels, data reported with statistically significant benefit for genomically guided therapy (p < .05); light blue panels, data reported with trend toward significance for genomically guided therapy (p < .10); orange, data reported with statistically worse outcome with genomically guided therapy.

Tests: F1, FoundationOne®; F1+F1H, FoundationOne® and FoundationOne® Heme; GRD, Guardant360®; Mult, multiple assays that include tests other than F1 or GRD. Only tests used to manage patients are listed; additional tests examined for other purposes (eg, to measure concordance data) are not listed.

Design/comparison: G/uG, guided vs unguided therapy; G/SC, guided vs standard of care; M/uM, matched vs unmatched therapy; P, prospective; R, retrospective.

Cancer stage: A, advanced/metastatic; mA, mostly advanced; Mult, multiple stages; NR, not reported.

PFS (progression free survival): n Denotes very small N (n < 10) for 1 or more comparison groups.

OS (overall survival): i, indeterminate value (median not reached in 1 comparison group).

Actionable mutations: May include “potentially actionable” mutations.

Targeted therapy: (+), on-label; (-), off-label; (±), on- and off-label; plain gray panel, on/off-label not specified.

Clinical trials: Reported identifying clinical trials for subset of patients.

AEs: adverse events.

Affiliation: One or more authors with financial interests or advisory roles with test developers.

*Wheler et al analyzed 188 patients in detail but reported actionable mutations from all 339 patients. Sadaps et al analyzed 313 patients (of 600 total patients) who underwent treatment changes with or without genomic-driven therapy.
Figure 10. Number and Size of Studies Reporting on Health Outcomes

Abbreviations: n.s., not statistically significant; OS, overall survival; PFS, progression-free survival; Resp/stb dis, response or stable disease; Sig, significant; Tm to fail, time to treatment failure.

A: Bars display the number of studies reporting on each health outcome. Most studies reported on overall survival (10 studies) or progression-free survival (9 studies). Some studies reported on multiple outcomes, so total number represented is larger than the number of studies. Dark blue and light blue panels represent studies with statistically significant and borderline significant effects favoring NGS testing. Gray panels represent nonsignificant effects. See Table 1 and Appendix I for additional details and outcomes on individual studies.

B: Mean number of patients (± standard error) for studies reporting (left) or not reporting (right) significant or near-significant benefits with NGS testing for one or more health outcomes.

Figure 10A above depicts the number of studies from Table 1 reporting on important health outcomes (overall survival, progression-free survival, response or stable disease, and time to treatment failure) and stratified by statistical outcome. Most studies reported on overall survival or progression-free survival. Eight of 15 studies (53%) reported significant or borderline significant benefits in outcomes with use of NGS testing. All statistically significant and borderline significant impacts from NGS testing were favorable. These studies’ average size was significantly larger than that of the 7 studies reporting no significant or borderline significant differences (Figure 10B). We excluded the study by Singal et al84 from this analysis because the number of patients was an order of magnitude larger than that of the other studies. This study reported better overall survival for the matched therapy group. Significant or borderline significant benefits were reported in 4 of 10 studies (40%) reporting on overall survival, 5 of 9 (56%) reporting on progression-free survival, and 2 of 4 (50%) reporting on treatment response or stable disease.
Figure 11 depicts the most common genes with mutations reported in the included studies. When provided, we tallied the most common mutations leading to targeted treatments. If only the most common mutations overall were reported, we included these in the tallies. When only raw data were reported on mutations for each patient, we counted the 3 or 4 most commonly mutated genes. ERBB2, KRAS, and TP53 were the 3 most commonly cited genes in the studies included in this section.

**Figure 11. Common Mutations Reported in Studies of Guided vs Unguided Therapy**

Bar graph of number of comparative data studies reporting on different mutations. When available, we reported on the most frequently detected mutations. If not directly reported, we report the most frequently found 3 or 4 genes, if the data were available. In some cases, only actionable genes were provided, in which case we reported on the most common actionable genes. In these studies, ERBB2, KRAS, and TP53 were the genes most commonly found to have variants.
NGS Panels vs Single-Gene or Small Gene Panels

An important consideration in evaluating large NGS panel tests is to compare their impact on patient care against single-gene or smaller gene panel tests. Only 2 retrospective studies\(^94,349\) addressed this comparison, which examined patient database records, and only 1 of the 2 studies\(^94\) analyzed outcomes. The latter study,\(^349\) not reporting outcomes, would typically not be included in our analysis because it used research use–only tests as comparators, but we briefly mention it here due to the extreme lack of evidence on this comparison.

The retrospective cohort study by Presley et al\(^94\) used the Flatiron Health Database to compare the outcomes of patients with lung cancer who had undergone broad-based genomic sequencing (any multigene panel testing of more than 30 genes) or routine genetic testing of 1 or 2 genes: \(\text{EGFR and/or ALK}\) only. Patients (n = 5688) had received a diagnosis of stage IIIIB/IV or recurrent nonsquamous advanced NSCLC and had received first-line antineoplastic treatment. A minority of these patients (n = 875; 15.4%) received broad genomic sequencing. The primary outcome was mortality at 12 months from the start of first-line treatment. Broad-based genomic sequencing was performed before third-line treatment with 12-month follow-up from the start of first-line treatment while routine testing was performed at any time. Survival at 12 months between the 2 groups was not significantly different with instrumental variable (41% chance of death for genomic sequencing vs 44.4% for routine testing) or for propensity score matching (42% vs 45.1% for genomic sequencing and routine testing, respectively).

Vail et al (2020)\(^349\) analyzed records of 480 solid tumor samples obtained from the Foundation Medicine database. Samples were collected at a single medical center over 4 years and were tested with the FoundationOne\(^\text{®}\) test’s 315-gene version. Records of sequence variants obtained from this test were compared with 161-gene and 50-gene versions of a research-use-only test (Ion Oncomine\(^\text{™}\), ThermoFisher Scientific) to determine which ones would, in principle, have been captured by the smaller tests. While the medium and small panels, unsurprisingly, found only a subset of the 2072 variants detected by FoundationOne\(^\text{®}\) (65.3% and 35.5%, respectively), the medium-sized panel would have identified all 318 patients with a clinically actionable variant.

Direct Comparisons of Different NGS Panel Tests

Weiss et al (2015)\(^346\) compared actionable biomarkers and turnaround times of FoundationOne\(^\text{®}\) with the Paradigm Cancer Diagnostics PCDx\(^\text{™}\) test. Tumor tissue samples (formalin fixed, paraffin embedded) from 21 patients with various solid tumor cancers were submitted for testing with both assays. The most common cancer types were thoracic (n = 7), gastrointestinal (n = 4), and urogenital (n = 3). The 2 tests were used to identify clinically actionable targets for commercially available drugs or drugs in clinical trials. PCDx\(^\text{™}\) reported more clinically actionable targets for commercially available drugs than did FoundationOne\(^\text{®}\). However, PCDx\(^\text{™}\) includes additional measures, such as gene expression levels (a measure of a gene activity), not assayed by the FoundationOne\(^\text{®}\) test. Notably, at the time the study was published, 5 authors of the Weiss et al study were current or former members of Paradigm Diagnostics, developer of the PCDx\(^\text{™}\) test.
3. Single-Arm Studies

Overview of Evidence

We identified and included 77 single-arm studies that explored the use of NGS testing for treating patients with cancer. Single-arm studies using emerging technologies and association studies are analyzed in separate sections of the report. We accepted studies that reported at least clinically actionable mutations and identified 77 studies that reported on one of the following outcomes: (1) patient health outcomes (14 studies),62,127,163,165,170,174,217,222,298,333,356,358,359,365 (2) patient management decisions/changes (17 studies),156,161,162,164,166,168,169,172,176,177,180,182,184,187,207,211,223 or (3) actionable mutations only (46 studies).175,178,179,181,183,185,186,188-190,202-204,206,213-215,218,219,224,225,228,230,328,329,332,334,336,340,342-345,348,349,353-355,357,359-361,363,364,366,367 Most studies reported on patients with advanced or metastatic cancers, and 16 of 77 studies also included patient ethnicity information.

Summary of Findings

Number and Size of Studies by Cancer Type and NGS Test

By cancer types, many single-arm studies enrolled patients with multiple cancer types (24 studies), indicating a tissue-agnostic approach for NGS testing (Figure 12). The “other” category includes gastric, basal cell carcinoma, and carcinoma of unknown primary site (see Appendix J). Median number of patients ranged from 45 to 3476 across all studies. Although certain cancers had high median enrollment, the data stemmed from 1 or 2 studies (eg, leukemia = 2 studies and prostate = 1 study) with high patient enrollment.
Figure 12. Study Count and Size Stratified by Cancer Type for Single-Arm Studies

The y-axis represents the number of studies identified focusing on a particular cancer. Each bubble’s center point aligns with the respective number of studies on each cancer type. Bubble size represents the median number of patients in the studies within a given cancer type (the legend depicts bubble sizes for studies of 30, 300, and 1000 patients). See Appendix J for list of “other” cancer types. For “Multiple” category, patient inclusion was not limited to one specific cancer type.

We also stratified studies according to the commercially available NGS test used. Many studies reported on the exclusive use of the FoundationOne® (25 studies) or Guardant360® liquid biopsy (17 studies; Figure 13). Other tests included CANCERPLEX® (Kew), OmniSeq® Comprehensive (OmniSeq), and JAX ActionSeq™ (The Jackson Laboratory).
Figure 13. Study Count and Size Stratified by Test for Single-Arm Studies

Bubble plot of study number and size by NGS test. The y-axis represents the number of studies using each given NGS test; each bubble’s center point aligns with the respective number of studies using that test. Bubble size represents the median number of patients in the studies within a given cancer type. The legend depicts bubble sizes for studies of 25, 75, and 150 patients. Most frequently used tests are plotted individually. The “multiple tests” category denotes studies that used 2 or more tests. See Appendix K for a list of “other” tests.

Abbreviations: F1, Foundation One; F1 CDx, FoundationOne® CDx solid tumor test; F1 Heme, FoundationOne® Heme test for hematologic malignancies.

Quantifying Evidence by Cancer Type and Health Outcomes

We examined the amount of evidence, in terms of the number of studies and patients, for each cancer type and major outcomes reported (actionable mutations, management changes, and health outcomes). Health outcomes included overall survival, progression-free survival, treatment response, and stable or progressive disease. We present a series of heat maps to summarize these data.

Figure 14 depicts a heat map of study count by common cancer types and health outcomes reported. The most numerous studies with key outcomes of interest were those reporting on patients with different cancer types, breast cancer, lung cancer, or “other” individual cancers (see Appendix J for cancer types in the “other” category). We identified no studies of bladder, kidney and renal pelvis, uterine, and thyroid cancers that met our criterion and reported outcomes of interest. Most studies reported on actionable mutations or on management decisions; fewer studies reported on health outcomes. Figure 15 follows the same conventions just described but quantifies evidence in terms of the number of patients. The number of patients with reported outcomes (tallied in the heat map) is fewer than the total study population because outcomes for all patients were not provided. Rough concordance exists between these data and those on study number, aside from the large studies reporting on leukemia.
Figure 14. Study Count Stratified by Cancer Type and Reported Outcomes for Single-Arm Studies

<table>
<thead>
<tr>
<th>Health outcomes</th>
<th>Breast</th>
<th>Lung</th>
<th>Prostate</th>
<th>CRC</th>
<th>Melanoma</th>
<th>Bladder</th>
<th>Kidney and RP</th>
<th>Uterine</th>
<th>Leukemia</th>
<th>Pancreas</th>
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</table>

Abbreviations: CRC, colorectal cancer; RP, renal pelvis.

This heat map displays the number of studies on each cancer type reporting on each of 3 outcomes (see far left panel). The number of studies for each cancer type/outcome combination is plotted within the cell, and darker shades represent more studies. For “Multiple,” studies included patients with different cancer types. For “Other” category, studies reported on a cancer type not listed in the figure (see Appendix J for full list).

Figure 15. Number of Patients Stratified by Cancer Type and Outcome Reported for Single-Arm Studies

<table>
<thead>
<tr>
<th>Health outcomes</th>
<th>Breast</th>
<th>Lung</th>
<th>Prostate</th>
<th>CRC</th>
<th>Melanoma</th>
<th>Bladder</th>
<th>Kidney &amp; RP</th>
<th>Uterine</th>
<th>Leukemia</th>
<th>Pancreas</th>
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</table>

Abbreviations: CRC, colorectal cancer; RP, renal pelvis.

Heat map of the number of patients reported by cancer type and outcome measure. Numbers depicted are the patients with reported outcome in each study and not total study population (the study on pancreatic cancer reported outcomes for only a small subset of patients). Other conventions are similar to those of previous figure: The number of patients is plotted in each cell, with darker shades representing more patients.
Figure 16 and Figure 17 are heat maps showing the quantity of evidence in study numbers and patient numbers, respectively, by individual test and reported outcome. Data are shown for the most frequently studied commercial NGS tests in our literature base (see Figure 3B). FoundationOne®, Guardant360®, and other tests had the most reported studies and patients with outcomes of interest. This series of figures shows that, irrespective of cancer type or NGS test used, most studies reported on actionable mutations, with fewer studies reporting on critical measures of clinical utility, such as management decisions and health outcomes.

**Figure 16. Count of Single-Arm Studies Stratified by Test and Reported Outcomes**

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<tr>
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<th>Caris</th>
<th>Clonoseq (original)</th>
<th>F1 CDx</th>
<th>F1 Heme</th>
<th>F1 Liquid</th>
<th>Guardant 360</th>
<th>Oncomine Dx</th>
<th>OncoPanel</th>
<th>OncoSeq</th>
<th>Tempus</th>
<th>Multiple</th>
<th>Other</th>
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<td>Health outcomes</td>
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</table>

Abbreviation: F1, FoundationOne®.

This heat map displays the number of studies by test reporting on each of 3 outcomes. The number of studies for each test/outcome combination is plotted within the cell, and darker shades representing more studies. For “Multiple,” 2 or more tests were used for the study population. For “Other,” studies reported on other tests not listed in the figure (see Appendix K for full list).
Figure 17. Number of Patients Stratified by Test and Outcome Reported for Single-Arm Studies

<table>
<thead>
<tr>
<th>Health outcomes</th>
<th>Caris Clonoseq</th>
<th>F1 (original)</th>
<th>F1 CDx</th>
<th>F1 Heme</th>
<th>F1 Liquid</th>
<th>Guardant 360</th>
<th>Oncomine</th>
<th>OncoPanel</th>
<th>OncoSeq</th>
<th>Tempus</th>
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</table>

**Abbreviation:** F1, FoundationOne®.

Heat map of the number of patients with reported outcomes stratified by the test used in the study. Conventions are similar to those of the previous figure. Numbers depicted are the patients with reported outcome in each study and not total study population. The number of patients for each test/outcome combination is plotted within the cell, and darker shades representing more patients. The vast majority of patients were tested with FoundationOne® solid tumor test (F1 original), FoundationOne® Heme (F1 Heme; for hematologic cancers), or Guardant360® assays.

**Quantifying Evidence From Single-Arm Studies by Study Design and Health Outcomes**

Most single-arm studies (80%) are retrospective in design (Figure 18A), and most reported only on actionable mutations without any report of subsequent management decisions or health outcomes (Figure 18B). A minority of studies reported on more critical outcome measures related to clinical utility (management changes or health outcomes). We note that studies reported on actionable mutations in different ways, and some common types of outcomes reported include the number of patients with ≥1 actionable mutation identified, the number of actionable mutations across all patients in a study, and the percentage of patients with actionable mutations.
We examined in more detail the 14 studies\textsuperscript{62,83,127,163,165,170,174,217,222,298,333,356,358,365} that reported on patient health outcomes following NGS-guided treatment (see Appendix L for detailed summaries of these studies that also reported on actionable mutations and targeted therapies used to treat patients). These studies enrolled patients with different cancers, including lung, melanoma, gastric cancer, and mesothelioma. The most frequently used NGS tests in these studies were FoundationOne\textsuperscript{®} and Guardant360\textsuperscript{®}. Targeted therapies included $\textit{BRAF}$ inhibitors, $\textit{mTOR}$ inhibitors, and other tyrosine kinase inhibitors. Duration of symptom improvement, response, and stable disease varied from 26 weeks to a maximum of 3 years with some patients ongoing.

Fourteen studies reported on 160 patients with one of the following outcomes: overall survival, stable disease, progression-free survival, response, and progressive disease. Only 2 of these 14 studies (14.3%) reported on overall survival or progression-free survival ($n = 40$).\textsuperscript{127,217} Arshad et al\textsuperscript{217} reported overall survival of 78% and progression-free survival of 46% in 39 patients with metastatic gastrointestinal stromal tumor 12 months after NGS testing. Patients in this study were treated with tyrosine kinase inhibitors based on ctDNA findings using Guardant360\textsuperscript{®}. The second study by Ugurluer et al\textsuperscript{127} reported on one patient with advanced malignant mesothelioma treated with vorinostat (off-label use) who survived 14 months after therapy initiation.

The remaining 12 of 14 studies (85.7%)\textsuperscript{62,83,163,165,170,174,222,298,333,356,358,365} reported on treatment response, symptom improvement, or progressive disease in patients after receiving targeted therapy based on identified alterations. Notably, 1 of the 14 studies (7.1%) used TMB to guide therapy and reported outcomes. Goodman et al\textsuperscript{356} reported high TMB in 4 patients with metastatic basal cell carcinoma, identified by FoundationOne\textsuperscript{®}, who were subsequently treated with PD-1 blockade therapy. Three patients (75%) achieved complete response or partial response, and one patient had progressive disease.

Figure 19 depicts the number of patients reported on for each of 3 different groups of health outcomes followed in these studies. Note that less direct measures, such as response, stable disease, or symptom
improvement, were reported for most patients. More critical measures of clinical utility, such as overall survival and progression-free survival, were reported for far fewer patients.

**Figure 19. Number of Patients by Reported Health Outcomes in Single-Arm Studies**

![Bar graph of the number of patients in single-arm studies with reported health outcomes.]

Abbreviations: OS, overall survival; PD, progressive disease; PFS, progression-free survival; SD, stable disease; sym imp, symptom improvement.

**Tumor Mutation Burden**

We also identified 9 studies that reported on TMB. Studies reporting on TMB classified patients into low, high, or intermediate TMB status. Higher TMB predicts favorable response to anti-PD1 or PD-L1 inhibition in solid tumors. In June 2020, FDA approved pembrolizumab for treating adult and pediatric patients with unresectable or metastatic solid tumor with high TMB (defined as ≥10 mutations/megabase) identified by an FDA-approved test. To date, FoundationOne® is the only FDA-approved companion diagnostic to identify patients with high TMB for treatment with pembrolizumab. Most patients in these 9 studies had low or intermediate TMB status. Only one study, by Goodman et al, reported that most patients (7 out of 8) with locally advanced or metastatic basal cell carcinoma had high TMB.
4. Concordance of Liquid Biopsy and Tissue-Based Tests

We identified 45 studies that compared liquid biopsy with tumor tissue sequencing and reported on clinical validity measures, such as concordance, sensitivity, and specificity. Guardant360® (30 studies) was the most frequently used assay, followed by FoundationACT™ (4 studies) and FoundationOne® Liquid CDx (3 studies; Figure 20). FoundationACT™ is a predecessor to FoundationOne® Liquid CDx. Other tests included InvisionFirst®-Lung and InVisionSeq™. Most studies on liquid biopsy tests reported on patients with lung and breast cancer and on cohorts of patients with different cancers (Figure 20). We note one systematic review on use of liquid biopsy in NSCLC that reported on positive percentage agreement with tissue-based testing for certain genes frequently mutated in NSCLC, including ALK, EGFR, and KRAS. While this review included 5 of the studies we reported on above, it also included studies on research use–only, custom, and noncommercial assays. For this reason, we did not include results from this review in our analysis.

Of the 45 studies, we identified 22 that reported comprehensive data on concordance in mutations identified using liquid biopsy and tumor tissue sequencing in the same patients. Of the 45 studies, we also identified 4 (2 studies on multiple cancer types, one on pancreatic, and one on NSCLC) that reported comprehensive data on liquid biopsy’s sensitivity for detecting alterations using tissue sequencing as the reference standard, and the sensitivity ranged from 25% to 85%. Two of the 4 studies also reported on specificity, which was greater than >95% in both studies. One additional study on colorectal cancer reported a positive percentage agreement of 76% between liquid biopsy and tumor sequencing, which increased to 100% when considering only those samples for which the time between liquid biopsy and tumor sequencing was fewer than 30 days. Twenty studies we surveyed were not included in this analysis either because they did not report clinical validity measures, such as concordance, sensitivity, or specificity, or reported values for only one or a few genes or for a subset of patients.

Figure 21 depicts the number of studies reporting percentage concordance between tissue-based and liquid biopsy tests, within different ranges of values. There is wide variation in values reported. Fifteen of the 22 studies (68.2%) reported concordance of 60% or greater. Importantly, only 6 of 22 studies reported concordance of 90% or more in identified mutations between the 2 methods. Many studies (n = 4; 18.2%) reported values of 30% or less. Of the 22 studies, 9 reported on the median duration, or a range of durations, between the 2 samples (tissue and blood), which ranged from 13 to 608 days. Rough negative correlation existed between concordance and the duration between the 2 sampling methods, but the relationship was not statistically significant. However, this might be due to the few studies analyzed.

Studies of lung and colorectal cancer reported higher concordance values than did studies of other cancers. All 13 studies in these 2 cancer types reported concordance of at least 60%. Due to the few included studies, and the highly variable time intervals between different sample collections (concordance tends to decrease over time as the cancer’s mutational profile changes), this conclusion remains tentative. Other factors that can influence concordance rates apart from cancer type include number and type of alterations that can be commonly detected using both methods, primary or metastatic tissue used for biopsy, study size, and performance of the specific tests used. Additional studies are required to analyze the relative contribution of each of these factors to concordance values.
Figure 20. Study Count Stratified by Cancer Type and Liquid Biopsy Test Used

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<th>Breast</th>
<th>Lung</th>
<th>Colorectal</th>
<th>Bladder</th>
<th>Kidney &amp; RP</th>
<th>Pancreas</th>
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<td>0</td>
<td>1</td>
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<tr>
<td>Other tests</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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</tr>
</tbody>
</table>

Abbreviation: RP, renal pelvic.

Heat map of the number of studies on each cancer type employing a specific test (see far left panel). Darker shades represent larger numbers of studies. For “Multiple,” studies included a mix of patients with different cancer types. For “Other,” studies reported on a cancer type not listed in figure (see Appendix J for full list). One systematic review covering several individual studies is not included in this map, so total studies add to 44.

Figure 21. Distribution of Studies by Reported Concordance of Liquid Biopsy and Tumor Tissue

Abbreviation: RP, renal pelvis.

Bar graph displays number of studies of different cancer types (colored panels) within each range of concordance. For “Multiple,” patient inclusion was not limited to a specific cancer type. For “Other,” studies reported on a cancer type not listed in the figure (see Appendix J for full list).
5. Modeling Studies of Effectiveness

Overview of Evidence

We found 6 studies exploring NGS testing’s cost effectiveness for treating patients with cancer.\(^{233-236,239,242}\) We limited inclusion to studies that constructed models based on, or that analyzed data from, the US health care system. We also limited our scope to studies comparing use of large NGS panel tests vs use of single-gene or other alternative testing strategies. We examined 4 studies of hypothetical patient populations\(^{233,235,236,239}\) and 2 studies analyzing real-world data from US claims databases.\(^{234,242}\) Publication years for included studies ranged from 2015 to 2020. Five of the 6 studies\(^{233-236,242}\) focused on patients with advanced NSCLC, while one modeled treatment of patients with metastatic melanoma.\(^{239}\) Four of 6 studies compared NGS panel testing vs serial single-gene testing,\(^{234,236,239,242}\) while the remaining 2 compared FoundationOne\(^{®}\) to a mix of conventional molecular diagnostic testing and smaller NGS hotspot panels\(^{235}\) to multiple alternative approaches, including single- or small gene panels (eg, \(ROST\) and \(BRAF\)) or serial single-gene testing (\(KRAS\) first followed by sequential tests of other genes).\(^{235}\)

Summary of Findings

Reported outcomes were primarily economic comparisons between NGS panels and the comparator test strategy; however, our analysis focuses on NGS testing’s reported clinical impact. Additional data summaries that include economic data from these studies are presented in Appendix M.

Most studies did not specify a particular NGS test they used in their models. Signorovitch et al\(^{233}\) based their analyses solely on the FoundationOne\(^{®}\) test. The test developer, Foundation Medicine, sponsored this study. Li et al\(^{239}\) modeled their analysis on a comparison between the 34-gene Oncovantage™ NGS panel and the cobas® BRAF V600 single-site mutation test.
Figure 22. Effectiveness of NGS Tests Estimated From Real-World Data and Modeling Studies

Bar graph summarizes effectiveness by study type (color), cancer type, and presence or absence of clinical benefit. Five of 6 studies indicated a clinical benefit for NGS testing. However, only 1 of 2 studies based on actual insurance claims data reported clinical benefit for NGS tests compared with comparators. The symbol \( \square \) denotes clinical benefit.

Figure 22 depicts the distribution of studies focused on lung cancer (5 studies) and melanoma (one study). Most studies (4 studies) employed hypothetical cohorts of patients (blue panels), while 2 studies analyzed US payer claims data (orange panels). Four of these 5 studies focused on lung cancer, while one modeled results for patients with metastatic melanoma.\(^{239}\) Five of 6 studies\(^{233,236,239}\) reported one or more clinical advantages (smiley faces) for NGS panel testing.

Two studies on lung cancer found that NGS testing provides clinical benefits, such as prolonged therapy and survival\(^{233}\) or identification of more patients for targeted therapies or clinical trials.\(^{236}\) Two other studies comparing NGS multigene panel testing against sequential single-gene testing with melanoma,\(^{239}\) and to sequential single-gene, exclusionary testing or hotspot panels,\(^{235}\) reported more identified targetable genetic variants with multigene testing. The fifth study,\(^{234}\) based on claims data from the Flatiron Health database, reported a slightly higher rate of targeted therapy assignment with multigene panel testing (21% vs 19% for single-gene testing) and slightly higher expected survival with multigene testing (1.2 vs 1.14 life-years for single-gene testing).

6. Associational Studies

Nineteen studies fit our criteria for associational studies.\(^{67,82,85,89,92,98,109,113,118,121,131,139,144,145,150,152-154,216,227}\) Most of these studies were retrospective and explored associations between mutations or other biomarkers (eg, homologous recombination deficiency [HRD]) and outcomes of patients treated with particular targeted therapies (eg, poly ADP-ribose polymerase [PARP] or PD-1/L1 inhibitors) or other treatments (eg, chemotherapy). Some were controlled trials testing the efficacy and safety of targeted therapies,\(^{152,153}\) but we categorized them as association studies because they were retrospective in design regarding examining the relations between genomic testing results and patient outcomes. Other studies were single...
arm and attempted to find associations between established NGS test-derived biomarkers and patient outcomes\textsuperscript{118,154} or to identify additional putative biomarkers for drug response, either performing new genetic analysis on archived tissue of patients who had completed trials\textsuperscript{92} or performing further post hoc analysis on previous genomic testing results.

Because of the tangential relationship of many of these studies to our report’s focus, we did not treat this class of studies in detail. We do note 4 studies conducted in support of the FDA-approved myChoice\textsuperscript{®} CDx Test (Myriad Genetics) for determining eligibility of patients with ovarian cancer for the PARP inhibitors, olaparib and niraparib.\textsuperscript{92,152-154} Three of these studies focused on the test’s intended patient population and purpose,\textsuperscript{152-154} while the fourth\textsuperscript{92} was exploratory. The myChoice\textsuperscript{®} test identifies patients with HRD-positive status because of deleterious mutations in BRCA\textsubscript{1} and BRCA\textsubscript{2} or positive genomic instability score (≥42 on a scale of 0-100). Two of these studies were RCTs,\textsuperscript{152,153} with only one reporting positive findings: that olaparib plus maintenance bevacizumab delayed cancer progression in patients with HRD-positive tumors but not in those with HRD-negative tumors.\textsuperscript{152} The other RCT\textsuperscript{153} reported that maintenance therapy with niraparib improved progression-free survival in all patient groups, regardless of HRD status. The single-arm study by Moore et al\textsuperscript{154} suggests that overall survival did not differ statistically between patients whom myChoice\textsuperscript{®} classified as HRD-positive or HRD-negative.

7. Emerging Technologies

Here we provide a separate review of studies that explored the use of newer, emerging NGS-based sequencing technologies. An exhaustive review of these topics is beyond the scope of this report; however, we present a brief overview of the studies captured in our literature search. Because most of these technologies are still in research or experimental stages, as opposed to routine clinical use, we did not limit inclusion to commercially available tests. We discuss strengths and weaknesses of these applications of NGS technology in the context of more mainstream commercial applications and provide some thoughts as to likely impacts and challenges of these technologies as they enter clinical use.
Whole-Exome Sequencing and Whole-Genome Sequencing

Our searches for NGS-based technologies affecting patient management in oncology indications identified 13 studies reporting on the use of WES\textsuperscript{15,16,18,263,271,282,287,298,302,307,311,315,320} and one study reporting on the use of WGS\textsuperscript{272} (Table 2). These studies reported on genetic correlations with treatment effect (2 studies), identification of clinically actionable mutations exclusively (9 studies), treatment changes guided by WES/WGS (one study), and health outcomes of patients managed with genomic testing (2 studies). All were single-arm studies lacking a comparator group.

Studies reporting on treatment change guided by WES/WGS or health outcomes of patients managed with genomic testing were generally testing the feasibility of integrating WES/WGS into a clinical setting. Lee et al\textsuperscript{15} performed both WES and targeted sequencing on solid tumors from 15 patients. Compared with targeted sequencing, WES reportedly identified pathogenic somatic variants in more cases (73% vs 53%). Findings reportedly informed cancer management in 66% of cases, with 20% of patients receiving a molecularly matched therapy and 47% referred to a familial cancer center. Bai et al\textsuperscript{287} performed WES on solid tumors from 953 patients and identified at least one clinically actionable mutation in 88.56% of patients. In a subset of 22 patients with actionable findings from WES, patients who received WES-directed therapy ($n = 11$) had improved progression-free survival (median 12 months) compared with patients ($n = 11$) who did not receive WES-directed therapy (median 4 months). Reda et al\textsuperscript{325} successfully performed WES on solid tumors from 456 of 506 patients, 75% of whom received at least one WES-based therapeutic recommendation. Among the patients with treatment recommendations, 23.1% ($n = 79$) were treated with a WES-based therapy, and 56.7% ($n = 149$) received standard of care. No difference in progression-free survival was observed between the 2 groups (2.5 vs 2.4 months).
Table 2. Whole-Exome Sequencing and Whole-Genome Sequencing Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Technology</th>
<th>Cancer type</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Studies reporting on genetic correlations with treatment effect</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xue et al 2020(^{263})</td>
<td>WES</td>
<td>Hepatitis B–associated hepatocellular carcinoma</td>
<td>38</td>
</tr>
<tr>
<td>Robinson et al 2015(^{311})</td>
<td>WES</td>
<td>Medulloblastoma</td>
<td>31</td>
</tr>
<tr>
<td><strong>Studies reporting only clinically actionable mutations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Allen et al 2014(^{16})</td>
<td>WES</td>
<td>Solid tumors</td>
<td>511 (clinically actionable findings reported for only a minority of patients)</td>
</tr>
<tr>
<td>Jones et al 2015(^{18})</td>
<td>WES</td>
<td>Solid tumors and hematologic malignancies</td>
<td>815</td>
</tr>
<tr>
<td>Schulze et al 2015(^{315})</td>
<td>WES</td>
<td>Hepatocellular carcinoma</td>
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<tr>
<td>Thomsen et al 2016(^{307})</td>
<td>WES</td>
<td>Bladder cancer</td>
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</tr>
<tr>
<td>Chow et al 2017(^{220})</td>
<td>WES</td>
<td>Nasopharyngeal carcinoma</td>
<td>10</td>
</tr>
<tr>
<td>Ghazani et al 2017(^{302})</td>
<td>WES</td>
<td>Colorectal cancer/non–small cell lung cancer</td>
<td>165</td>
</tr>
<tr>
<td>Priestley et al 2017(^{272})</td>
<td>WGS</td>
<td>Solid tumors</td>
<td>2520</td>
</tr>
<tr>
<td>Pectasides et al 2018(^{298})</td>
<td>WES</td>
<td>Gastroesophageal adenocarcinoma</td>
<td>28</td>
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<tr>
<td>Bertelson et al 2019(^{271})</td>
<td>WES</td>
<td>Solid tumors</td>
<td>636</td>
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<tr>
<td>Liu et al 2019(^{282})</td>
<td>WES</td>
<td>Squamous cell lung cancer</td>
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<td><strong>Studies reporting on genetic correlations with treatment effect</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Novak et al 2015(^{326})</td>
<td>WES</td>
<td>Diffuse large B-cell lymphoma</td>
<td>51</td>
</tr>
<tr>
<td>Robinson et al 2015(^{311})</td>
<td>WES</td>
<td>Hepatitis B–associated hepatocellular carcinoma</td>
<td>38</td>
</tr>
<tr>
<td>Xue et al 2020(^{263})</td>
<td>WES</td>
<td>Medulloblastoma</td>
<td>31</td>
</tr>
<tr>
<td><strong>Studies reporting on patient management changes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lee et al 2018(^{15})</td>
<td>WES</td>
<td>Solid tumors</td>
<td>15</td>
</tr>
<tr>
<td><strong>Studies reporting on patient health outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bai et al 2019(^{287})</td>
<td>WES</td>
<td>Solid tumors</td>
<td>953 (patient outcomes reported for only a minority of patients)</td>
</tr>
<tr>
<td>Reda et al 2020(^{325})</td>
<td>WES</td>
<td>Solid tumors</td>
<td>506</td>
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</table>

Abbreviations: WES, whole-exome sequencing; WGS, whole-genome sequencing
Multiomics

Our searches for NGS-based technologies affecting patient management in oncology indications identified 24 studies reporting on the use of multiomic/integrated genomic profiling (Table 3).256,258,266,269,277,280,281,283,289,292,295,296,299–301,305,308,312,316,318,321,323 Most of these studies (23 studies) reported on the combined use of WES or WGS with RNA-seq. In a subset of these studies, DNA and RNA-seq were supplemented by epigenetic profiling (eg, DNA methylation analysis; 3 studies), comparative genomic hybridization (CGH; one study), or microarray-based gene expression analysis (one study). One study reported on the combined use of WGS and proteomic analysis. Studies reported on a range of outcomes. Four studies reported exclusively on the identification of clinically actionable mutations. Five studies reported on genetic correlations with treatment effect, including 3 studies on genetic variants assessing response to treatment, one study looking at genetic variants associated with progression to more aggressive disease, and one study on genetic variants associated with risk of recurrence after surgery. Five studies reported on treatment modifications. Ten studies reported on health outcomes of patients managed with multiomic testing, 6 of which reported on response rates and 4 of which reported on progression-free survival or duration of remission.

Multiomic approaches to cancer treatment are currently limited primarily to academic centers with a strong focus on biologic discovery, in addition to cancer management. While coming years are likely to see increased use in these settings, broader adoption will likely require the development of key predictive markers that drive uptake. For example, adoption of targeted genomic sequencing in NSCLC has been driven by the discovery of multiple predictive genetic variants (eg, EGFR, ALK, ROS, MET) that has rendered using a multigene panel approach more efficient than using single-gene approaches. Analogously, if multiple protein or RNA markers predict response for a specific cancer type, then this could promote adoption of a proteomic or transcriptomic approach to NSCLC testing. Alternatively, multiomic testing could be driven by identifying protein expression, RNA expression, or epigenetic signatures predictive of drug response that are identifiable only through a multiomic approach (rather than single protein/RNA/epigenetic event). This would be analogous to how identifying TMB as a predictive marker for response to immune checkpoint inhibitor therapy, and identifiable only using a broad sequencing approach, may drive adoption of multigene panels and WES/WGS.
<table>
<thead>
<tr>
<th>Study</th>
<th>Technology</th>
<th>Cancer type</th>
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<tr>
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<td>Lier et al 2018</td>
<td>WES, RNA-seq</td>
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<td>Ogura et al 2018</td>
<td>WES, RNA-seq, DNA methyl.</td>
<td>Myxofibrosarcoma</td>
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<td><strong>Studies reporting on genetic correlations with treatment effect</strong></td>
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<tr>
<td>Wong et al 2018</td>
<td>WGS, RNA-seq</td>
<td>Liver metastases from pancreatic neuroendocrine tumors</td>
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<td>Awad et al 2020</td>
<td>WES, RNA Seq</td>
<td>Chronic myeloid leukemia</td>
<td>59</td>
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<tr>
<td>Facchinetti et al 2020</td>
<td>WES, RNA-seq, CGH, targeted panel</td>
<td>Non–small cell lung cancer</td>
<td>8</td>
</tr>
<tr>
<td>Li et al 2020</td>
<td>WES, WGS, RNA-seq</td>
<td>Gastric cancer</td>
<td>35</td>
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<tr>
<td>Miyanga et al 2020</td>
<td>WES, RNA-seq, microarray-based gene expression</td>
<td>Pulmonary carcinoid tumors</td>
<td>25</td>
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<td><strong>Studies reporting on patient management changes</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Oberg et al 2016</td>
<td>WES, RNA-seq</td>
<td>Pediatric solid tumors and hematologic malignancies</td>
<td>101</td>
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<td>Uzilov et al 2016</td>
<td>WES, RNA-seq</td>
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<td>46</td>
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<td>Aguirre et al 2018</td>
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<td>Frank et al 2019</td>
<td>WES, RNA-seq</td>
<td>Glioblastoma</td>
<td>30</td>
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<tr>
<td>Khater et al 2019</td>
<td>WES, RNA-seq</td>
<td>Pediatric solid tumors and hematologic malignancies</td>
<td>84</td>
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### Studies reporting on patient health outcomes

<table>
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<th>Study</th>
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<tbody>
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<td>Mody et al 2015⁹¹⁶</td>
<td>WES, RNA-seq</td>
<td>Pediatric solid tumors and hematologic malignancies</td>
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<tr>
<td>Borad et al 2016¹²³</td>
<td>WES, WGS, RNA-seq</td>
<td>Solid tumors</td>
<td>35</td>
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<tr>
<td>Worst et al 2016¹⁰⁸</td>
<td>WES, WGS, RNA-seq, DNA</td>
<td>Pediatric solid tumors and hematologic malignancies</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>methylation analysis, microarray-based gene expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Harttrampf et al 2017³⁰¹</td>
<td>WES, RNA-seq, targeted panel</td>
<td>Pediatric solid tumors</td>
<td>75 (outcomes reported for only a small percentage of patients)</td>
</tr>
</tbody>
</table>

### Studies reporting on patient health outcomes

<table>
<thead>
<tr>
<th>Study</th>
<th>Technology</th>
<th>Cancer type</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koschmann et al 2018⁴⁰⁰</td>
<td>WES, RNA-seq</td>
<td>Glial brain tumors</td>
<td>52</td>
</tr>
<tr>
<td>Pishvaian et al 2018²⁹³</td>
<td>WGS, proteomic</td>
<td>Pancreatic cancer</td>
<td>640 (outcomes reported for only a small percentage of patients)</td>
</tr>
<tr>
<td>Singer et al 2018²⁹²</td>
<td>WES, WGS, RNA-seq</td>
<td>Solid tumors</td>
<td>22 (outcomes reported for only 11 patients)</td>
</tr>
<tr>
<td>Tuxen et al 2018³¹⁸</td>
<td>WES, RNA-seq</td>
<td>Solid tumors</td>
<td>591</td>
</tr>
<tr>
<td>Pfaff et al 2019²⁷⁷</td>
<td>WES, RNA-seq, DNA methylation analysis</td>
<td>Diffuse intrinsic pontine glioma</td>
<td>21 (outcomes reported for only 8 patients)</td>
</tr>
<tr>
<td>Tsang et al 2019³⁸³</td>
<td>WGS, RNA-seq</td>
<td>Non–small cell lung cancer</td>
<td>26</td>
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</tbody>
</table>

**Abbreviations:** CGH, comparative genomic hybridization; RNA-seq, RNA sequencing; WES, whole-exome sequencing; WGS, whole-genome sequencing.

### Disease Monitoring and Minimal Residual Disease Detection

Our searches identified 14 studies that reported on NGS-based detection of MRD or disease recurrence (Table 4). Of these 14 studies, 3 used a commercially available MRD assay to monitor the efficacy of experimental treatment regimens in hematologic malignancies. The remaining 11 studies used a variety of assays to assess disease recurrence or MRD in either hematologic malignancies (5 studies) or solid tumors (6 studies) using either personalized (8 studies), nonpersonalized (2 studies), or commercially available (one study) assays. These studies reported on the difference in time points between molecular disease monitoring/MRD detection and conventional disease monitoring (5 studies) or correlations between molecular disease monitoring/MRD detection and patient outcomes (6 studies).

Use of ctDNA as a marker of disease progression is likely to increase in coming years, particularly in patients with solid tumors. The technology underlying ctDNA assessment is also being used in liquid biopsy applications to perform genetic testing of cancers to predict treatment sensitivity. Potential exists for synergy between these approaches to drive adoption of ctDNA-based approaches in both the predictive genetic test and disease monitoring spaces.
Table 4. Disease Monitoring and Minimal Residual Disease Detection

<table>
<thead>
<tr>
<th>Study</th>
<th>Technology</th>
<th>Cancer type</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Studies reporting on disease monitoring/MRD to measure depth of response to therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ryan et al 2016&lt;sup&gt;306&lt;/sup&gt;</td>
<td>MRD using commercially available assay (clonoSEQ&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>Chronic lymphocytic leukemia</td>
<td>11</td>
</tr>
<tr>
<td>Ruan et al 2018&lt;sup&gt;290&lt;/sup&gt;</td>
<td>MRD using commercially available assay (clonoSEQ&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>Mantle cell lymphoma</td>
<td>10</td>
</tr>
<tr>
<td>Alonso et al 2020&lt;sup&gt;260&lt;/sup&gt;</td>
<td>MRD using commercially available assay (clonoSEQ&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>Myeloma</td>
<td>139</td>
</tr>
<tr>
<td>Studies reporting on disease monitoring/MRD to predict disease progression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Van Wezel et al 2014&lt;sup&gt;314&lt;/sup&gt;</td>
<td>MRD using a personalized assay</td>
<td>Neuroblastoma</td>
<td>8</td>
</tr>
<tr>
<td>Ivey et al 2016&lt;sup&gt;310&lt;/sup&gt;</td>
<td>MRD using a nonpersonalized assay</td>
<td>Acute myeloid leukemia</td>
<td>346</td>
</tr>
<tr>
<td>Chaudhuri et al 2017&lt;sup&gt;26&lt;/sup&gt;</td>
<td>MRD using a personalized assay</td>
<td>Lung cancer (localized)</td>
<td>40</td>
</tr>
<tr>
<td>Hirsch et al 2017&lt;sup&gt;304&lt;/sup&gt;</td>
<td>MRD using a personalized assay</td>
<td>Acute myeloid leukemia</td>
<td>69</td>
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<tr>
<td>Shin et al 2017&lt;sup&gt;303&lt;/sup&gt;</td>
<td>MRD using a commercially available assay (LymphoTrack&lt;sup&gt;®&lt;/sup&gt; IGH)</td>
<td>Acute lymphoblastic leukemia</td>
<td>8</td>
</tr>
<tr>
<td>Kim et al 2018&lt;sup&gt;284&lt;/sup&gt;</td>
<td>Recurrence monitoring using a nonpersonalized assay</td>
<td>Acute myeloid leukemia</td>
<td>104</td>
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<tr>
<td>Balagopal et al 2019&lt;sup&gt;284&lt;/sup&gt;</td>
<td>MRD using a nonpersonalized assay</td>
<td>Acute myeloid leukemia</td>
<td>30</td>
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<td>Kim et al 2019&lt;sup&gt;276&lt;/sup&gt;</td>
<td>Recurrence monitoring using a personalized assay</td>
<td>Gastric cancer (localized)</td>
<td>25</td>
</tr>
<tr>
<td>Azad et al 2020&lt;sup&gt;268&lt;/sup&gt;</td>
<td>MRD using a personalized assay</td>
<td>Esophageal cancer (localized)</td>
<td>45</td>
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<tr>
<td>Bratman et al 2020&lt;sup&gt;322&lt;/sup&gt;</td>
<td>Recurrence monitoring using a personalized assay</td>
<td>Solid tumors (advanced)</td>
<td>94</td>
</tr>
<tr>
<td>Hellmann et al 2020&lt;sup&gt;267&lt;/sup&gt;</td>
<td>Recurrence monitoring using a personalized assay</td>
<td>Non–small cell lung cancer</td>
<td>24</td>
</tr>
</tbody>
</table>

Abbreviation: MRD, minimal residual disease.
Other Studies

Our searches identified 2 studies that used NGS-based methods to identify cancer neoantigens.\textsuperscript{261,279} Neoantigens (also known as tumor-specific antigens) are proteins produced by genetic variants that have occurred in cancer cells and, therefore, differentiate tumor cells from normal tissues through immunologic and genetic recognition. Neoantigens are unique to individual patients’ tumors and may serve as a biomarker for likelihood to respond to immunotherapies or serve as potential antigens in therapeutic cancer vaccines. Fang et al \textsuperscript{261} reported on the use of WES to identify neoantigens from solid tumors to be used in personalized peptide therapeutic cancer vaccines, and Chen et al \textsuperscript{279} reported on the use of a targeted sequencing panel to identify neoantigens from solid tumors to be used in personalized dendritic cell vaccines. Both studies reported on patient outcomes from a few patients enrolled in single-arm studies.

Our searches identified one study that used data from an NGS-based targeted sequencing panel to characterize a spectrum of mutations associated with a microsatellite instability phenotype in endometrial cancer.\textsuperscript{291} The presence of microsatellite instability in cancers is associated with increased response to immunotherapy with checkpoint inhibitors; therefore, evaluation of NGS panel data for microsatellite instability could be used to identify patients appropriate for immune checkpoint inhibitor therapy without the need for separate microsatellite instability testing.

8. Ongoing Clinical Trials

Our searches identified 23 ongoing clinical trials with direct relevance to evaluating applications of NGS panel tests for evaluating or treating patients with cancer. We did not include studies in which the primary goal was to evaluate targeted therapies or other treatments. We limited our selection to trials that specified use of a commercially available NGS panel test and those listed as actively recruiting patients, enrolling patients, or ongoing but not actively recruiting patients.

Cancer types covered in these trials include breast cancer (3 trials), colorectal cancer (2 trials), gastrointestinal cancers (one trial), leukemia (2 trials), lymphoma (2 trials), lung cancer (6 trials), myeloma (one trial), prostate cancer (2 trials), and multiple solid tumor or other cancers (4 trials). Most of these trials are using (or plan to use) FoundationOne® CDx (7 trials), Guardant360® (7 trials), clonoSEQ® (5 trials), or Oncomine (5 trials). The purposes of these trials include using NGS tests to characterize the mutational profile of various cancer types, guiding selection of targeted therapies, measuring impacts on outcomes, screening for metastasis, establishing disease prognosis, measuring MRD or tumor mutation burden, characterizing disease progression or response with ongoing therapy, and measuring concordance between liquid biopsy and solid tumor assays.

Three prospective comparative cohort studies on this list, studies of Oncomine or Guardant360®, will address a critical evidence gap by comparing management decisions and health outcomes for patients managed with NGS testing vs those managed with standard strategies (ie, without information from genomic sequencing). Most of these trials are enrolling, or plan to enroll, patients with metastatic NSCLC. One additional trial studying use of the Signatera test (Natera, Inc) on patients with colorectal cancer has a secondary objective of determining genomic testing’s impact on patient quality of life. Also notable is the single-group observational trial using the FoundationOne® CDx test to explore genomic profiles of Korean patients with breast cancer. This objective is driven by potential differences in the molecular profile of breast cancer in patients of Asian origin compared with their Western...
counterparts. This study may also compare outcomes of patients with molecularly guided therapy vs historical controls.

Two trials\textsuperscript{370,373} plan to compare blood-based genomic profiling with the Guardant360\textsuperscript{®} test vs results from standard tissue-based testing. These studies may contribute evidence for assessing concordance in variant detections between liquid biopsy and tumor tissue testing.

See Appendix N for a complete list of trials with hyperlinks to their ClinicalTrials.gov profiles.

9. Stakeholder Input

As discussed in the Methods section, we conducted semistructured phone interviews with numerous key informants to garner input on our report’s scope and important issues related to the use of NGS-based genomic testing in cancer management. We selected key informants who provided a range of perspectives, including clinicians (medical oncologists), patients, patient advocates, payers, and public policymakers (see Methods section for general procedural details and Appendix B for a list of guiding questions for phone interviews).

Here we provide a short summary of some topics and discussion points raised during these interviews. Rather than providing an exhaustive survey of these discussions, we have focused on the topics most pertinent to the literature we surveyed. We revisit some of these topics and discuss them in the context of our findings in the Discussion section.

Use of NGS Panels for Informing Treatment

- There was consensus from the clinical experts that use of genetic testing to find single targets specifying targeted therapies is standard of care. The question is, for a given condition, whether a single-gene test or a larger multigene NGS panel is appropriate. Clinical experts also mentioned the relatively minor cost of testing compared with the large cost of targeted therapies.

- Clinicians opined that they accept the technology’s importance, but they want to see a chain of evidence linking genetics to drug choice and to therapeutic response. If that chain of evidence is clear, they are willing to accept that NGS tests can improve patient outcomes, despite a lack of direct evidence showing that NGS tests have a positive impact (assuming that the analytic validity of the included single-gene tests is adequate).

- One medical oncologist stressed that tissue-agnostic treatments (ie, larotrectinib for \textit{NTRK} fusions and pembrolizumab for TMB-high solid tumors) have increased adoption of NGS panels in clinical practice.
Discussion of Evidence Gaps in Assessing NGS Testing’s Clinical Utility

- In discussing the ideal evidence for assessing clinical utility of NGS panel tests, clinical experts raised concerns about the difficulty of carrying out RCTs comparing genomically guided patient management vs management without genomic testing. Such studies are outlined by genetic testing evidence frameworks, such as EGAPP.3
- Barriers cited were the number of different platforms and tests to investigate and the large variation in specific genetic makeup of tumors, even within a given cancer. This latter concern presents a serious challenge for achieving balance in a randomized study. Therefore, trials would need to be very large and probably focus on a single cancer type. Performing such studies for different cancer types with the many available tests would be infeasible.
- Several stakeholders—clinical experts, payers, and patients—commented on uncertainties surrounding analytic validity of individual biomarkers in NGS-based genomic tests, particularly for ctDNA (ie, liquid biopsy) tests.
- One stakeholder suggested a repository for analytic validity data for commercial NGS tests that could be overseen, vetted, and updated as results on new actionable genes become available or if assay platforms or approaches should change.

Determining “Actionability” of Biomarkers

- One clinical expert commented on efforts to classify genetic variants according to actionability (ie, their value for guiding treatment). Many approaches have been explored to assign biomarkers to different “tiers” of actionability based on evidence for their impact on patient care.
- In this approach, biomarkers (eg, gene variants) that inform efficacy or safety of FDA-approved targeted therapies (assayed by approved companion diagnostic tests) would be classified in the highest tier, followed by successively lower tiers for biomarkers that have less clinical evidence, or only biological plausibility (lowest tier).

Insurance Coverage of Testing

- Clinicians and payers both commented that coverage for testing and subsequent therapies is generally provided when justified by evidence. This is particularly true for companion diagnostic tests associated with FDA-approved targeted therapies.
- The primary concern regarding testing is whether to cover serial single-gene testing or a panel test. While some conditions clearly require a panel test (eg, lung cancer), single-gene testing may be appropriate for other conditions.
- One patient advocacy representative raised concerns about the lack of coverage for more than one NGS test (as cancer genetic profiles can change over time, sometimes becoming resistant to therapies). Though a recent CMS national coverage determination on NGS testing specifies a single use for most cases (instituted to guard against investigational or unnecessary repeat testing that does not improve outcomes), CMS may revisit this policy, particularly as more evidence becomes available regarding the impacts of measures, such as MRD and disease monitoring, which depend highly on NGS testing.
- Clinicians and payers both agreed that coverage is not provided for drug treatments with biologic rationales but no direct evidence.
Communication

- Clinicians remarked about the challenges of communicating results to patients.
- Some patients do not understand the distinction between genetic testing that informs treatment and that which probes ancestry or racial and ethnic background. For this reason, some patients are hesitant to sanction this analysis.
- Stakeholders also raised concern about the need to standardize terminology to ensure clarity in communication between clinicians and patients.

Health Care Disparities and Other Barriers to Using NGS Testing

- At least one representative from each stakeholder group expressed concern about the access to genomic testing for specific populations of patients.
- Stakeholders were concerned about access to, and awareness of, these technologies in underserved populations, including African American, Hispanic, and Native American communities and within rural and small community settings.
- Stakeholders also expressed concerns about the knowledge and training of clinicians serving these populations. Stakeholders mentioned that many clinicians may not be aware of genomic testing’s benefits for their patients or are not trained to select appropriate tests or interpret their results to guide treatment.
- Socioeconomic status affects educational background, which, in turn, can influence patient knowledge and awareness of options that new technologies and treatment options could provide.

This last point was illustrated by one of our patient informants who, by virtue of her family experience and educational background, leveraged resources and peer-group interactions through a patient advocacy organization to identify an ongoing clinical trial in her home region testing a new medication targeted to her specific mutation. Importantly, her oncologist was not aware of this treatment option. The immense availability of information from varied sources makes possible important discoveries by willing and motivated patients that can positively affect their care. But such serendipity favors those fortunate enough to have the upbringing, education, and time to participate in planning their own care.
Discussion

Below, we first broadly discuss our report’s major findings regarding the evidence addressing NGS’s impact on management and health outcomes of patients with cancer. We discuss the major identified evidence gaps (topics or questions for which published clinical evidence is lacking), offer some putative explanations for these gaps, and propose approaches to addressing them. Finally, we propose topics for future research and some goals to guide these efforts.

In addition to summarizing findings from the clinical literature, we discuss concerns and perspectives from our key informants that pertain to our findings and conclusions (see Appendices B and D for additional information on methods and our approach to stakeholder interviews, respectively). We intended these interviews to help identify the regulatory, clinical, and contextual issues centered on genomic sequencing results and their impact on managing patients with cancer and their health outcomes. These interviews identified barriers to or facilitators for the use of genomic sequencing to guide cancer management.

General Summary of Findings

Even with this considerably circumscribed evidence review, limited to widely available commercial tests, we included more than 300 individual studies covering use of 33 unique commercial NGS multigene panel tests in our literature base. Single-group, retrospective studies, generally regarded as low-quality designs, were most prevalent among included studies. Studies reporting the number or percentage of patients with clinically actionable mutations and/or management decisions were most common. A smaller subset of these studies reported influences on patient health outcomes. Fewer yet reported on specific treatments patients received and the biomarkers that guided these decisions.

Comparative studies of patients managed with NGS multigene testing vs those undergoing standard management (eg, physician choice not guided by genomic sequencing) were scarce, representing only about 5% of total included studies. Importantly, none of these studies were RCTs, the accepted gold-standard study design for determining an intervention’s efficacy. About one-half of these comparative studies showed a significant impact of NGS testing on patient outcomes, such as overall survival (OS), progression-free survival (PFS), or treatment response. Significant differences in group outcomes, when reported, indicated a benefit from NGS testing.

The FoundationOne® solid tumor and the Guardant360® liquid biopsy tests (offered by Foundation Medicine and Guardant Health, respectively) are the 2 most frequently researched tests in our literature base. Considerably less research has focused on other commercially available NGS multigene tests. Notably, studies enrolling a mix of patients with different cancers were common and outnumbered the studies focused on any single cancer type. This may reflect a shift in emphasis to tissue-agnostic screening, with focuses on characterizing a tumor’s genomic profile regardless of tissue of origin. This approach facilitates patient recruitment and may also spur research on off-label molecularly guided therapies by identifying patients possessing the established biomarkers but in different tissues of origin than those specified in drug approvals. Breast, lung, and colorectal cancer are represented most heavily in the literature, while much less evidence focuses on bladder, kidney, uterine, and thyroid cancers and leukemia. Studies of patients with lung cancer most often reported on patient health outcomes, possibly because of the many biomarkers and FDA-approved therapies available for lung cancer. Lung cancer was also represented heavily in the small subset of studies with comparative data. This may reflect bias in the selection of cancer types most likely to be affected by use of multigene panel tests and may, therefore,
skew evidence from these studies toward greater overall impact for multigene panel testing. Several common cancers, most notably prostate and melanoma, had very few studies. This is somewhat surprising given that they are the third and fifth most common cancers, respectively, according to the National Cancer Institute SEER Program. Prostate cancer and leukemia, despite having few studies, have many patients in each study, likely due to the high prevalence of these cancers. Other factors, such as availability of FDA-approved therapies and the market lifetime of therapies (ie, how long they have been on the market), may affect the representation of certain cancers in the literature.

Commercial NGS technologies for cancer treatment currently consist primarily of targeted gene panels. These tests assay a specific, circumscribed set of genes, varying from a few to several hundred. Our literature review found a substantial evidence base for research studies of WES and WGS. These NGS-based techniques sample much larger expanses of the genome and therefore can detect many more genetic variants than can standard targeted panel tests. Though these technologies are, at present, largely employed for research purposes, we believe their uptake into clinical use will advance in the coming years.

In research studies, WES and WGS are increasingly used with RNA sequencing, gene expression and protein analysis, and epigenetic profiling (such as DNA methylation, an endogenous biochemical modification that inactivates genes). Studies of these so-called multiomic approaches, similar to research on commercial NGS panel tests, report on clinically actionable mutations and patient treatment decisions as well as on patient health outcomes.

MRD, the presence of macroscopically undetectable cancer following successful treatment, is emerging as a measure of interest in using NGS technologies for disease monitoring. NGS-based assessment of MRD has already diffused into clinical care for certain hematologic malignancies. While targeted NGS panels are likely better suited for MRD detection in patients expected to have low levels of ctDNA (eg, patients with nonmetastatic cancers), WES- and WGS-based approaches may detect novel (de novo) mutations or mutations specific to small subclonal cell populations within the initial tumor that are undetectable with targeted methods. These advances are all likely to further NGS technology’s use in guiding cancer treatment. We will address the strengths, weaknesses, and expected impact of these technologies below.

Evidence Gaps

Comparative Studies Assessing Clinical Utility of NGS Panel Tests Are Lacking

Much has been written about the unique challenges of evaluating evidence on genetic testing (eg, NASEM, 2017). As discussed in the introduction, multiple evidence frameworks for genetic testing have emphasized 3 crucial domains: analytic validity, clinical validity, and clinical utility. Evidence for clinical utility—the impact of testing on patient care and health outcomes—has understandably been most valued. However, we found that direct evidence supporting a positive impact of NGS-based multigene testing on patient outcomes is severely limited. Only about 5% of our included studies provided data comparing health outcomes of patients managed with or without genomic testing, or comparing those receiving genomic-guided therapy vs those managed with unguided therapy. Importantly, none of these studies were RCTs; rather, they were comparative cohort or single-arm studies with post hoc comparisons. Findings from a recent systematic review by the CMS supporting its national coverage determination on NGS testing for cancer management were in general agreement with these conclusions. While RCTs investigating this question are challenging to undertake (see discussion below), clearly a more general paucity of comparative data exists for assessing clinical utility.
Our analysis went beyond identifying a shortage of these studies. We also presented data suggesting that small- to moderate-size enrollments in some of these studies may have been responsible for failure to detect statistically significant impacts of NGS testing. Studies reporting one or more statistically significant, or near-significant, benefits of NGS testing on patient health outcomes had significantly larger cohorts. This was true even when excluding the largest study, with more than 4000 patients, that reported a significant positive impact on OS. Therefore, our analysis underscores not only a shortage of comparative studies, but also often insufficient statistical power to detect advantages conferred by testing. Particularly for studies that pool data from patients with different cancer types into single cohorts, substantial variations in the condition, stage of disease, treatment history, and subsequent treatment decisions, as well as other variables, such as demographics, could all influence whether benefits from testing are observed. Therefore, conducting studies of sufficient size and statistical power to detect NGS testing’s benefits is critical. Direct comparisons of NGS multigene panel testing to alternative testing strategies, such as single-gene tests or small gene panels, were even rarer. We found only 2 such studies, only one of which reported on a health outcome (ie, OS), for which no significant benefit was found with NGS multigene panel testing. Identifying the conditions and variables that favor testing with large multigene panels over alternative strategies is an important consideration for both payers and providers and a question worthy of further research effort.

Finally, only one study provided a direct comparison between 2 different NGS multigene panel tests, and the study was published by the index test developer. Any concerted effort to compare the effectiveness of different multigene panel tests would require a massive and sustained effort, as new tests enter the market and existing tests undergo expansions to their gene panels and refinement of their laboratory protocols. Such research efforts may not be feasible and may not be valued by the clinical community or other stakeholders. A crucial factor in any comparative assessment of NGS panel tests would include assessments of the analytic and clinical validity of individual biomarker assays (see below for more on this issue). Beyond this, meaningful differences favoring one multigene panel test over another would likely involve variations in the individual biomarkers assayed and ancillary considerations, such as turnaround time and clarity of the reported results. These are not variables that can be approached in a timely way by large, carefully conducted comparative studies.
Documenting Impacts of NGS Testing on Clinical Decisions

Documenting associations between identified variants and the ensuing therapy choices they motivated is critical to evaluating clinical decisions and their impact on patient care. Unfortunately, this information was often unavailable. While decisions could sometimes be inferred based on the use of approved drug therapies, the wealth of information generated by NGS panel testing not only directs clinicians to FDA-approved indications, but may also motivate off-label treatment. Off-label therapies are drugs that have undergone preclinical and clinical testing to confirm safety and effectiveness, but are used outside their indicated drug labeling use (eg, osimertinib is approved for use in patients with NSCLC whose tumors have specific EGFR variants; a possible off-label use would be for patients with colorectal cancer). We sought to identify evidence on the effectiveness of off-label drug use based on NGS testing. We considered off-label use of any FDA-approved drug used for a variant outside its labeling (within the same gene or in different genes), any drug used in a different cancer type, or any drug used for a different cancer stage. While several comparative studies report the percentage of patients treated with off-label drugs, we identified no studies permitting comparisons of the comparative effectiveness of on- and off-label treatments. Importantly, biomarkers considered to be actionable in one cancer may not be in another cancer type, even if the underlying molecular pathway is identical. This lack of evidence, therefore, represents a significant gap and an important opportunity for future research. Establishing the extent to which data can be extrapolated from one cancer type to another would prove useful to clinicians and payers.

Many single-group studies are still reporting only on actionable mutations identified with no documentation of management decisions in response to, or outcomes resulting from, test results. Reporting actionable biomarkers, alone, provides, at best, very indirect evidence for NGS testing’s clinical impact. At this stage of the field’s maturity, it is unclear what further purpose such studies serve. To be more informative, studies should report critical outcomes, such as OS and PFS, as well as adverse events. Reporting on adverse events, in particular, was often lacking.

Analytic Validity of NGS Tests

Finally, establishing the analytic validity of NGS panel tests is a major concern raised by stakeholders. The accuracy and reliability with which a test detects a given genetic variant of interest provides a foundation upon which clinical validity and clinical utility depend. While analytic validity of tissue-based NGS testing generally appears to be well accepted, stakeholders raised concerns about liquid biopsy tests. Data on analytic validity are usually not published and could vary over time as laboratory assays and procedures undergo change and refinement. Our analysis of concordance data for liquid biopsy and solid tumor tests showed variable results across studies. Evidence maps of these data suggest that concordance between the 2 tissue types may vary with the cancer type, but other explanations are possible. For instance, the time lag between collection of tissue and blood samples can influence concordance due to ongoing changes in a cancer’s mutational profile over time. Both samples should be collected at the same time, but this is often not done. Therefore, as the intervening time between solid tissue and blood sample collections increases, one might expect a decrease in concordance of detected mutations. Analysis of a subset of 9 of our included studies that reported the median number of days between tissue and blood samples found a rough negative correlation between intersample time and concordance in detected mutations. This relationship fell short of statistical significance, but this is not surprising given the relatively few studies. Other factors, such as tumor heterogeneity and clonal hematopoiesis, could...
also introduce discrepancies between tissue- and blood-based testing. In general, efforts to provide more readily available data on analytic validity seem warranted. Further research with higher-quality studies that minimize the time between solid tissue and blood-based sampling would be helpful to investigate other potential sources of discrepancy between solid tumor and liquid biopsy tests.

Our Findings in Context of Current Clinical Practice

Despite very limited formal evidence, support for NGS testing is established in the clinical community and will likely only increase. This conclusion was echoed in discussions with multiple stakeholders for this project. One of our subject matter experts commented that failure to provide genomic sequencing to patients with lung cancer, a condition for which numerous actionable genes and choices of targeted therapies exist, would be ethically indefensible. The acceptance of this technology despite very limited direct supportive evidence likely stems from the wealth of evidence on the effectiveness of targeted therapies and their associated companion diagnostic (typically single-gene) tests, coupled with acceptance of the analytic validity of NGS tests.

As the number of actionable biomarkers and associated targeted therapies continues to grow, the demand for NGS-based genomic testing will likely increase. As detailed in the Introduction, CMS recently issued a national coverage determination on NGS testing, the general guidelines of which are extended or qualified by local coverage determinations for specific cancers or treatment contexts. These advances have accompanied FDA approvals or clearances of NGS-based genomic panels, both for solid tissue and blood samples (eg, FoundationOne® CDx).

Evidence of buy-in from multiple stakeholder groups indicates that NGS-based targeted genomic profiling is an accepted and valued tool for managing patients with cancer. This technology’s decreased cost and increasing availability will likely support this trend through the next 5 years. During this time, larger-scale NGS techniques (eg, WES/WGS) and multiomics testing (including immunohistochemistry, mRNA, and gene expression analysis together with mutational profiling) will likely gain increased acceptance and application. In fact, we already see multiomic testing products, such as PCDx™ (Paradigm Diagnostics), paving the way for this latter approach. Use of WES and WGS may begin to encroach on targeted sequencing applications, particularly if measures such as tumor mutation burden become more widely adopted. A comprehensive survey of the impact of these newer, wider-scope sequencing techniques, and the quantitative measures derived from these applications, are potential topics for future systematic review projects.

Despite widespread use, challenges exist in routine implementation of NGS testing, and the aforementioned technology advancements will likely exacerbate these challenges. Remaining current in the rapidly evolving field of precision medicine is challenging for clinicians, and this requires awareness of NGS testing’s appropriateness for a specific patient as well as its potential utility. Other operator factors, such as training and incorporating results into the electronic medical record (EMR), may affect the extent to which NGS can be used effectively. In the future, NGS test ordering capabilities may integrate completely into EMR software, which would remove a significant barrier and facilitate testing. Panel tests, even those with established clinical utility and accuracy of individual biomarker tests, are not necessarily the best management option for all patients. Contextual considerations, such as the number of relevant biomarkers for the patient’s condition (ie, are there enough options to warrant a multigene panel test rather than single-gene testing?), must be evaluated. Sufficient evidence of this type might identify...
conditions and circumstances in which NGS testing is preferable to single-gene approaches, and vice versa.

Complementary Approaches for Evaluating Clinical Utility

The scarcity of trials comparing management with NGS multigene panel tests vs suitable comparators (single-gene testing or unguided management) suggests that barriers may exist to conducting such studies, including the following:

- The difficulties and costs of conducting large RCTs or other types of comparative studies are considerable (eg, see discussion of challenges from the National Cancer Institute [NCI] MATCH study).380
- There is an immense challenge in achieving group balance in demographics and disease characteristics, such as different cancer types, the distribution of genetic variants across patients, stages and locations of disease, and the number and types of previous treatments.
- Since rare cancers may not be sufficiently represented,3 even in large cohorts, assessing NGS testing’s impact on these conditions is difficult with traditional approaches.
- The number of new actionable gene variants and targeted therapies increases over time with corresponding expansions in NGS panel tests, challenging the pace at which evidence from “ideal” clinical utility studies (such as those designated by EGAPP and other frameworks) can be provided.
- Clinicians generally accept and value NGS-based genomic profiling, making it difficult to find researchers willing to forgo this testing option for suboptimal strategies (eg, single-gene testing) when managing patients (particularly for aggressive conditions with numerous biomarkers and treatment options, such as lung cancer).
- Coverage decisions by CMS and commercial insurance companies indicate substantial consensus in the payer community for NGS technology’s value as a tool for guiding cancer treatment.
- Given all the above considerations, clinicians may not regard such comparative studies to be critical.

While efforts to assess the clinical utility of NGS multigene panel tests are in progress (eg, the NCI MATCH study), high-quality comparative trials, such as RCTs, are lacking and may not be forthcoming. Therefore complementary, albeit indirect, approaches to evaluating evidence for clinical utility could be considered. One approach could borrow from FDA’s Breakthrough Devices Program and its analysis of clinical validity for the FoundationOne® CDx test (F1 CDx). This evaluation involved (1) identifying individual biomarkers (eg, gene mutations, duplications, deletions) with established utility that are assayed by F1 CDx and (2) evaluating F1 CDx’s performance by comparing its detection of critical biomarkers (those with established utility) vs a reference standard, such as an FDA-approved or -cleared single-gene companion diagnostic test. If an NGS panel includes analysis of a gene variant with established utility, and the panel’s performance at identifying this variant is demonstrated to be satisfactory, then we may infer (by an indirect chain of evidence) that the panel has some level of clinical utility. Rigorous and overarching guidance for assessing the analytic validity of NGS panel tests has been described previously (eg, American College of Medical Genetics [ACMG] standards), and the extensive validation processes for the recent NCI MATCH study have been published.382
Figure 23 summarizes 2 complementary approaches for evaluating the clinical utility of NGS panel tests. Figure 23A diagrams the traditional methodologic, or test comparison, approach that begins with the test and evaluates its impact compared with that of no testing or other testing paradigms (eg, single-gene tests). This approach has been emphasized in evidence frameworks for genetic tests, including perhaps the most recognized EGAPP initiative, and advocates high-quality RCTs or cohort studies. Figure 23B depicts the complementary chain of evidence approach that borrows from FDA’s approach to approval of FoundationOne® CDx. In this approach, analysis begins with establishing biomarkers considered to have utility (such as those associated with FDA-approved targeted therapies). Supporting evidence for utility would demonstrate safety and efficacy of a targeted therapy for a subset of patients who can be identified reliably by the biomarker of interest. NGS panels containing those biomarkers are evaluated to measure the accuracy and reliability with which the biomarkers are detected, such as by measuring concordance of NGS test results for a given biomarker against a reference standard (typically single-gene) test, as was done for part of the FoundationOne® CDx approval process. Therefore, expanding research efforts to ensure that NGS panel tests accurately identify the biomarkers identified by FDA-approved companion diagnostic assays could provide indirect evidence for clinical utility.

We stress that the chain of evidence approach could provide a complement to, not a replacement for, the standard methodologic, or test comparison, approach. Even for a panel including assays for multiple actionable biomarkers with high analytic validity, conclusive evidence for a positive impact on patient outcomes is not a given. For instance, a very large targeted gene panel (or perhaps a test employing WES or WGS) may provide a quantity of information that proves confusing for most clinicians (ie, increasing decisional uncertainty). This could in turn result in treatment errors or suboptimal treatment choices. Larger panels may also require a more robust bioinformatics pipeline, leading to longer turnaround times and potentially delaying treatment initiation. Clearly, there are complexities that the indirect, chain of evidence approach cannot address. Conversely, a strength of the chain of evidence approach is its emphasis on measuring performance of individual biomarker tests within the larger panel. Because a given NGS panel may have acceptable performance for one biomarker but not for another, the chain of evidence approach could elucidate weaknesses of an individual NGS panel that standard clinical utility studies of the test would not detect. This is of particular concern for liquid biopsy NGS panel tests, which was raised in our discussions with clinician and payer stakeholders. As additional biomarkers and associated FDA-approved therapies become available, research is needed to ensure that NGS panel tests accurately assay these new biomarkers. Therefore, both approaches have unique strengths and weaknesses. Research efforts employing both approaches would be ideal, particularly as panel tests increase in size and complexity.
Figure 23. Two Complementary Paths to Establishing Clinical Utility for NGS Panel Tests

Complementary approaches can be used to establish clinical utility of next-generation sequencing (NGS) genomic panel tests. A: The standard methodologic, or test comparison, model compares the health outcomes of patients managed with testing vs those managed without genomic testing or with serial single-gene testing. This can be achieved with large randomized trials, although nonrandomized comparative designs (eg, cohort studies) or post hoc comparisons of single-arm observational studies can also contribute evidence. We found relatively little evidence employing this model to examine commercial NGS tests, none of which was of high methodologic quality. B: The chain of evidence approach can provide indirect evidence for clinical utility by identifying actionable biomarkers (eg, EGFR for predicting response to gefitinib) included in NGS panel tests and testing their performance (diagnostic accuracy) against a reference standard (for instance, an FDA-approved companion diagnostic test, such as the Roche cobas® EGFR mutation test), similar to the model that FDA used to evaluate FoundationOne® CDx. Establishing that an NGS panel test accurately assays multiple individual biomarkers with agreed-upon clinical utility (such as those associated with FDA-approved targeted therapies) would constitute indirect evidence for the panel test’s clinical utility. The 2 models could provide complementary approaches for assessing clinical utility of NGS panel tests.

Implicit in the discussion of the chain of evidence approach is the flexibility it affords in assessing the utility of existing NGS panels when the included array of biomarkers is changed or expanded. In such circumstances, most stakeholders would presumably agree that it is unnecessary to conduct all new studies to demonstrate a multigene panel test’s utility, if such studies were performed previously or if other compelling evidence for the test’s utility has been accepted. In such situations, it is necessary only to evaluate the analytic and clinical validity of the new biomarkers as add-on components to the existing test. In this way, adding biomarkers with actionability or established utility can be regarded as adding incrementally to the test’s potential for clinical value.

It is important to note, however, that this same convention does not apply to other classes of tests, such as gene expression profiling tests and other assays that use statistical algorithms (eg, logistic regression or machine learning approaches) to derive profiling or risk scores on which to base treatment decisions (eg, to characterize a tumor as benign or malignant). For these tests, adding additional data to a panel of existing biomarkers mandates new studies or compelling analysis to demonstrate that the same algorithmic approach, or variation of it, matches or bests the performance of a test’s previous versions. Though such tests are outside the scope of this report, as genomic testing’s diversity and application continue to evolve and expand, it is important to agree on strategies that provide necessary but
reasonable criteria for acceptable evidence of utility, while also allowing for important innovations and refinement.

Approaches for Categorizing Actionability of Biomarkers

To our knowledge, there is no agreed-upon definition of an actionable variant. Perhaps unsurprisingly, we found references to clinically actionable mutations to often lack transparency and consistency across studies. Actionability was most frequently defined in terms of association with an FDA-approved targeted therapy or eligibility for a clinical trial. However, associations with off-label therapies and accordance with guideline recommendations were also cited as criteria. Clearly, actionability was ascribed to biomarkers with varying levels of supportive evidence. Making clear the criteria for defining actionability, and reasons for the particular choice, would facilitate comparisons of findings from different studies and could provide a more systematic approach for assessing the clinical utility of multigene panel tests.

In the above outline of the chain of evidence approach, we discussed biomarkers with known utility in terms of association with FDA-approved targeted therapies. Such biomarkers are sometimes categorized as tier 1 or level 1, denoting high-quality and/or extensive evidence supporting clinical utility. However, many other biomarkers have not reached this level of validation but may nevertheless have limited evidence for utility. These biomarkers can be said to have actionability, meaning that they inform reasonable treatment selections that might have positive impacts on patient outcomes. As research progresses, biomarkers once thought to be nonactionable (or without utility) may become actionable (or be ascribed utility) as their roles in disease onset and progression are established and new medications targeting these molecular pathways are developed. Given these issues, establishing an agreed-upon classification system for actionability based on the quantity and quality of supporting evidence at a given time might help clarify and provide context for study findings and provide an additional objective approach for analyzing the individual biomarkers included in NGS multigene panels.

Several approaches have been developed to evaluate the utility of individual biomarkers. For instance, FDA’s Center for Devices and Radiological Health (CDRH) provides a 3-tier system for evaluating the evidence supporting gene variants within NGS panel tests for cancer profiling. Table 5 provides a summary of this framework. This approach classifies individual biomarkers embedded within NGS tests according to the types and levels of supporting evidence, including clinical guidelines, analytic validity studies, clinical validity studies, and peer reviewed publications.
Table 5. Center for Devices and Radiological Health (CDRH) Approach to Reporting Biomarkers in NGS Tumor Profiling Tests

<table>
<thead>
<tr>
<th>Level of evidence designation</th>
<th>Type(s) of supporting evidence</th>
<th>Clinical implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Companion Diagnostics (CDx)</td>
<td>“Claims are supported by analytical validity of the test for each specific biomarker and a clinical study establishing either the link between the result of that test and patient outcomes or clinical concordance to a previously approved CDx.”</td>
<td>“CDxs are tests that provide information that is essential for the safe and effective use of a corresponding therapeutic product.”</td>
</tr>
<tr>
<td>2 Cancer mutations with evidence of clinical significance</td>
<td>“Claims are supported by a demonstration of analytical validity (either on the mutation itself or via a representative approach, when appropriate) and clinical validity (typically based on publically available clinical evidence, such as professional guidelines and/or peer-reviewed publications).”</td>
<td>Tests for biomarkers described as cancer mutations with evidence of clinical significance enable health care professionals to use information about their patients’ tumors in accordance with the clinical evidence, such as evidence presented in professional guidelines, as appropriate.”</td>
</tr>
<tr>
<td>3 Cancer mutations with potential clinical significance</td>
<td>“Claims are supported by analytical validation, principally through a representative approach, when appropriate, and clinical or mechanistic rationale for inclusion in the panel. Such rationales would include peer-reviewed publications or in vitro pre-clinical models.”</td>
<td>“These mutations may be informational or used to direct patients towards clinical trials for which they may be eligible.”</td>
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</table>

Two other notable examples of evidence frameworks are provided by the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT) and the OncoKB precision oncology knowledge base. The ESCAT framework aims to aid oncologists in prioritizing potential targets for clinical use by classifying targets for precision medicine according to evidence of clinical utility. The ESMO scale of actionability has 6 tiers (I, II, III, IV, V, X) detailing biomarker-drug combinations based on strength-of-evidence evaluations from clinical studies. The framework also aims to help clinicians therapeutically prioritize genomic alterations described in tumor profiling reports to decrease the chance for misinterpretation of results leading to missed opportunities for effective treatment or overinterpretation of hypothetical targets.

OncoKB takes the evidence hierarchy a step further by pairing it with a precision oncology knowledge base containing information regarding treatment implications of specific biomarkers. The knowledge base is organized hierarchically by gene, alteration, indication, and level of evidence and annotates the significance of somatic molecular alterations. The potential treatment implications are stratified by the level of evidence that a specific biomarker is predictive of drug response on the basis of FDA labeling, National Comprehensive Care Network guidelines, disease-focused expert group recommendations, and scientific literature. Biomarkers are designated as level 1, 2, 3A, 3B, 4, R1, or R2 and are broadly categorized as standard care, investigational, or hypothetical targets. A dedicated panel of physicians and cancer biologists review and edit biomarker-associated investigational therapeutic strategies. Knowledge bases, such as OncoKB, in principle, have potential to reduce clinician uncertainty. Appendix O summarizes the OncoKB and ESCAT frameworks in more detail, illustrating the different evidence tiers and their defining criteria.
These efforts provide models for new approaches to evaluating utility of individual biomarkers, beyond those associated with FDA-approved targeted drug therapies. These approaches can be extended to evaluating off-label uses for targeted therapies, a common practice among oncologists that has proved helpful to some patients. The major challenges in leveraging these developing approaches is to (1) provide comprehensive and transparent methodologies for determining variant actionability and the strength of evidence backing assessments, and (2) work toward classification schemes that achieve broad acceptance by clinicians, payers, and guideline developers. This aim could begin with a comprehensive analysis of existing approaches for classifying actionability and weighing their relative merits and limitations. Opportunity exists for guideline developers to play an integral role in this effort to establish standardized approaches to validating and tracking actionable variants and associated treatment options in a rigorous and fully transparent manner that provider and payer communities can agree on.

Future Impacts of Emerging Technologies

NGS technologies and their applications to cancer genomics continue to expand rapidly. While the number of genes in targeted panels continues to increase, WES and WGS now have a strong presence in clinical research. These technologies will likely advance into mainstream clinical practice. WES and WGS can detect many more genetic variants than standard targeted NGS panel tests, which carries both advantages and drawbacks. While these techniques may provide more information regarding pathogenic variants and disease state, the added information burden may increase confusion and uncertainty about the significance of many additional detected genomic variants. This information burden will likely require more investigation into use of these techniques in clinical practice as well as bioinformatics research to help clinicians properly leverage the additional information provided by these assays. Widespread adoption of these techniques in clinical practice also faces technical hurdles. WES and WGS typically require tissue samples of higher quantity and quality, and the potential for repeated tissue samples can increase burden and stress on patients.

The broader scope of genomic analysis is accompanied, necessarily, by reduced sequencing depth compared with targeted NGS. This may compromise the accuracy with which common actionable gene variants are identified and may preclude detection of subclonal genetic variants that are less prevalent among cancer cells but which may have important implications for treatment choice. WES and WGS currently have longer turnaround times compared with targeted sequencing due to increased sequencing complexity and bioinformatics burden to identify important variants in the larger expanse of information. Given the above considerations, we project that WES and WGS will provide complementary options for genomic profiling rather than supplanting targeted sequencing, at least in the near future.

NGS-based assessment of MRD has been widely adopted for MRD testing in certain hematologic malignancies. In coming years, we expect that assessment of solid tumor–derived ctDNA, through liquid biopsy tests, to gain wider use for detecting MRD and disease monitoring. Liquid biopsy testing’s major strength is its minimal invasiveness, requiring only a blood sample instead of solid tissue biopsy. The technology underlying ctDNA assessment is also being used in liquid biopsy applications to perform genetic testing of cancers to predict treatment sensitivity. However, as discussed above, questions remain about these tests’ analytic validity, as concordance rates in mutation detection with tissue testing can be highly variable. Further research could investigate these discrepancies and the implications these tests have for applications such as MRD.
Health Care Disparities and Barriers of Access to Genomic Testing and Treatments

Beyond evaluating the clinical utility of NGS tests, effort is needed to ensure that effective new technologies are accessible to all patients. Though our review did not research the effect of health care disparities, at least one representative from each stakeholder group (payers, providers, patients, and representatives of patient advocacy organizations) expressed concern about access to genomic testing in small community or rural health care settings and in minority communities. Underserved populations cited by stakeholders included African American, Native American, and Hispanic communities. Stakeholders also raised concern that the education, awareness, and training of most clinicians serving such communities are inadequate to leverage these new technologies, issues that have been raised in the literature (eg, Smith et al 2016389). Numerous stakeholders echoed similar concerns about proper training even for clinicians in larger urban health care centers, where familiarity with newer technologies is expected. Aside from increased need for formal training, stakeholders identified lack of clear and consistent use of terminology in the field of genetic testing as a source of confusion for both clinicians and patients and communication between them (see a recent white paper from the Consistent Testing Terminology Working Group374 for examples and suggestions for improvement). Finally, stakeholders also cited access to clinical trials as another factor driving disparities in care, especially in rural areas. Of course, with these problems come opportunities for additional research to identify limitations in the availability and uptake of these new technologies and for efforts to ensure that they are available to, and understood by, clinicians serving in small community or rural settings and underserved populations.

Concerns raised early in the development of genetic testing were that these technologies might exacerbate existing health care disparities (eg, see Suther and Kiros389). Some of these concerns trace to general disparities in socioeconomic standing and health care access. Artiga et al (2020)390 noted that African Americans were 1.5 times more likely to be uninsured compared with Whites between 2010 and 2018, and that the uninsured rate for Hispanics remained more than 2.5 times higher than that for Whites. While genomic testing’s cost continues to decline, these tests still represent a prohibitive expense for individuals lacking health insurance. Beyond cost coverage, systems are needed to widely disseminate information about genomic testing’s importance to vulnerable populations and to encourage uptake of these technologies by providers and patients in these communities.
Racial and Ethnic Disparities in Cancer Profiling

During our literature review and in our analyses of key comparative and single-arm observational studies of NGS tests, we found relatively few studies reporting on the racial and ethnic background of their patient populations. For instance, 16 of 77 (21%) single-arm observational studies on commercial targeted NGS genomic tests (excluding studies of newer technologies, such as WES and WGS) reported on their patients’ racial and ethnic backgrounds. Only 7 of 15 studies (47%) comparing guided vs unguided treatment reported on these data. Even when racial and ethnic backgrounds were reported, ensuing analyses often did not attempt to explore variations in cancer mutational profiles or treatment-outcome relationships across racial and ethnic backgrounds.

While most studies are of modest size and lack the statistical power to attempt such analyses, some evidence suggests there may be systematic differences in mutation profiles and disease aggressiveness across different races and ethnicities. Concern has been raised about underrepresentation of patients of minority groups in genetic databases and the potential impact of this limitation on the use of genomic data to effectively treat these patients. Importantly, any systematic variations may involve multiple convergent factors, which are difficult to dissociate. For instance, socioeconomic variations can affect lifestyle behaviors such as diet, smoking status, and physical activity, which can contribute to health disparities through environmental impacts on genetics and health (ie, epigenetic factors). However, having the requisite data to explore these dynamics is key.

Without focused effort to address data inequality, not all groups will benefit from discoveries in cancer genomics. Ongoing research efforts should prioritize inclusion of more diverse study populations with thorough reporting of patient demographics, including racial and ethnic background. Also, when statistical power permits, genomic data should be stratified to explore mutation profiles across racial and ethnic backgrounds and, even more importantly, treatment-outcome relationships. Addressing this crucial evidence gap requires prioritizing inclusion of racial and ethnic minorities in genomic databases. If any systematic differences exist in these data, it is important to characterize them so that genomic profiling data are used to their full potential in treating patients with cancer from all racial and ethnic backgrounds. This is a very important topic worthy of further investigation for individual clinical trials and, potentially, for systematic review.

Study Limitations

This review has several important limitations. First, we restricted our analysis to the most widely used commercial NGS multigene panel tests. Many additional published studies employing research use–only (RUO) tests (including RUO versions of commercially available tests) and a more limited literature on tests specific to single academic institutions or health care systems are available and may have contributed additional evidence. This is particularly noteworthy in light of the relative scarcity of comparative data for clinical utility. Also, some studies that used NGS-based multigene panels did so in conjunction with other testing methodologies, but NGS testing’s impact alone was not discernible from the results. Several such studies, excluded from our analysis, are summarized in tabular form in Appendix P. Obviously, some of the positive impacts of multiomic approaches were likely due to NGS technologies.

Perhaps most notably, our focus on studies of NGS testing excluded a vast literature on drug therapy trials that compared biomarker-driven vs non–biomarker driven treatment, including the use of single-gene companion diagnostic tests. Many large systematic reviews with meta-analyses have summarized
findings from such studies and found favorable impacts of biomarker-driven treatment (eg, studies by Schwaederle et al\textsuperscript{394,395} and Fontes Jardim et al\textsuperscript{396}). These studies did not specify NGS testing as an inclusion criterion and, in fact, included studies used mixed testing methods, of which DNA biomarkers were only one. However, each of these reviews included more than 100 individual studies, with the Schwaederle reviews including several hundred studies each. These numbers underscore the vast quantity of data providing indirect support for the utility of NGS multigene panels, assuming the panels have adequate analytic and clinical validity. Because this literature addresses drug trials, it contains detailed information about the efficacy and safety profiles of specific medications as applied to well-defined patient populations. This represents a wealth of detailed information that cannot reasonably be provided by idealized RCTs comparing patient management with and without the use of NGS testing. While this project’s limited timeframe made surveying this very extensive literature infeasible, future projects might examine a circumscribed portion of this evidence, perhaps limited to a select set of biomarkers or a specific cancer type, and explore how it might affect assessments of clinical utility of NGS technologies.

The search strategy we used to identify our literature base also had limitations. For instance, the studies supporting the myChoice\textsuperscript{®} HRD test (see Associational Studies in the Results section) are essentially drug trials that correlated myChoice\textsuperscript{®} classification of patient homologous recombination deficiency (HRD) status with health outcomes following treatment with PARP inhibitors. Since these studies did not focus on evaluating NGS testing’s impact, our formal searches did not capture them; rather, we identified them through knowledge of this specific test, its characteristics, and its intended purpose. Searches may have failed to capture other studies using commercial NGS tests to explore genetic variants underpinning treatment responses to targeted therapies, though we are not aware of any other such studies.

The overarching goal to provide a broad overview of NGS technology in cancer treatment precluded a more in-depth analysis of individual studies. For instance, we did not compile data for specific outcome measures across multiple studies, provide detailed values (eg, effect sizes), and formally rate the strength of evidence by outcome (eg, OS, PFS). However, given that most of the literature was of uniformly low quality, the value of more detailed analysis is questionable.

Finally, genomic testing exhibits rapid development in the number of tests coming to market or gaining approval, expanding gene panels of existing tests, and new biomarkers (eg, tumor mutation burden) and companion diagnostics. For example, FoundationOne\textsuperscript{®} CDx received additional companion diagnostic indications and Guardant360\textsuperscript{®} CDx received companion diagnostic approval by FDA during production of this report. Therefore, our literature search might have been limited to more recent studies (perhaps the past 5 years) to better detect the current impact of recent treatment advances (eg, targeted therapies) over what was possible several years ago. A shorter review could also slightly reduce the delay to publication. Though we feel justified in choosing a wider temporal scope for the broad overview aimed at for this project, the rapid change in this field clearly warrants consideration of future rapid review projects.

**Conclusion**

Our evidence map analysis exposed a quantitatively and qualitatively limited evidence base supporting the positive impact of commercially available NGS-based genomic sequencing tests for managing patients with cancer. The limitations in evidence may be due to the many challenges posed by traditional comparative studies of clinical utility, such as RCTs. However, clinical utility of multigene panel tests might be inferred by assessing the utility of individual biomarkers within the panel and validating that panel tests assay these biomarkers accurately. Though this latter approach is traditionally considered indirect
from a methodologic perspective, it might instead be viewed as a complementary approach that can provide additional important information that traditional studies of clinical utility cannot. Much work remains but realizing the full potential of NGS-based cancer genomic profiling is critical to continuing innovation in patient care.
References


77. Favazza LA, Parseghian CM, Kaya C, et al. KRAS amplification in metastatic colon cancer is associated with a history of inflammatory bowel disease and may confer resistance to anti-EGFR therapy. *Mod Pathol.* 2020;33(9):1832-1843. doi.org/10.1038/s41379-020-0560-x. PMID: 32376853


95. Fabrizio DA, George TJ, Dunne RF, et al. Beyond microsatellite testing: assessment of tumor mutational burden identifies subsets of colorectal cancer who may respond to immune checkpoint inhibition. *J Gastrointest Oncol.* 2018 9(4):610-617. doi.org/10.21037/jgo.2018.05.06. PMID: 30151257


375. National coverage analysis (NCA) tracking sheet for next generation sequencing (NGS) for Medicare beneficiaries with advanced cancer (CAG-00450R). Centers for Medicare and Medicaid Services. Accessed September 14, 2020. https://www.cms.gov/medicare-coverage-database/details/nca-tracking-sheet.aspx?NCAId=296&SearchType=Advanced&CovSelection=Both&NCSelection=NCA%5C+CAL%7CNC&D%7C+MEDCACY7C+TAC%7C+MCD&ArticleType=BC%7C+AD%7C+RTC%7C+Reg&PolicyType=Both&s=All&KeyWord=ngs+cancer&KeyWordLookUp=Title&KeyWordSearchType=And&kq=true&bc=EAAAABAAIAAA


Appendices

Appendix A: CMS Local Coverage Determinations for NGS Tests for Cancer Profiling

The table below provides a list of LCDs issued by the Centers for Medicare & Medicaid Services (CMS) at the time of this report's drafting. The number and identification numbers of policies change frequently, so the below list may not be current when this report is published. LCD policies can be accessed at CMS’s Medicare Coverage Database.

Table A-1. CMS Local Coverage Determinations on NGS Tests for Management of Patients with Cancer

<table>
<thead>
<tr>
<th>Title</th>
<th>Contractor</th>
<th>ID</th>
<th>Summary</th>
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<tbody>
<tr>
<td>Biomarkers for Oncology</td>
<td>Novitas Solutions, Inc</td>
<td>L35396</td>
<td>Covered indications include the following next-generation sequencing tests:</td>
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<td>- ThermoFisher Oncomine DX Target Test for Non–Small Cell Lung Cancer (NSCLC)</td>
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<td></td>
<td>- LungSeq®</td>
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<td>Limitations for all covered indications include this note:</td>
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<td>“Please refer to the indications for any restrictions specific to the various assays. Please see NCD 90.2 for coverage details related to next generation sequencing (NGS) for Patients with Advanced Cancer. Most genomic testing should be a once in a lifetime test. Documentation in the medical record should clearly support the need for repeat testing to include the following: recurrence of disease, change in behavior of disease, etc.”</td>
</tr>
<tr>
<td>Genomic Sequence Analysis Panels in the Treatment of Hematolymphoid Diseases</td>
<td>National Government Services, Inc</td>
<td>L37606</td>
<td>“Coverage Indications, Limitations, and/or Medical Necessity</td>
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<tr>
<td>Acute Myelogenous Leukemia (AML)</td>
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<td>Indications</td>
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<tr>
<td>Genomic Sequential Analysis Panel will be considered reasonable and necessary in the evaluation of blood or bone marrow samples in the following clinical circumstances:</td>
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<td>- Newly diagnosed patients with AML who are undergoing induction therapy, and who are suitable candidates for post-induction transplantation or consolidation therapy at the time of testing, and meet one of the following cytogenetic criteria:</td>
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<tr>
<td>o normal karyotype</td>
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<td>o core binding factor</td>
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<td>- Previously diagnosed patients with AML, who have not responded to induction chemotherapy, or who have progressed following induction. The patient</td>
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<td>Title</td>
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<td>must be a candidate for transplantation at the time of the testing.</td>
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<td>• Patients with AML, who have responded to treatment, either chemotherapy or transplantation, with evidence of relapse.</td>
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<td><strong>Myelodysplastic Syndromes (MDS)</strong></td>
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<td></td>
<td><strong>Indications</strong></td>
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<td>Genomic Sequential Analysis Panel will be considered reasonable and necessary in the evaluation of blood or bone marrow samples in the following clinical circumstances:</td>
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<td>• Patients with clinical signs or symptoms of myelodysplastic syndromes (MDS) or myelodysplastic/myeloproliferative overlap syndromes (MDS/MPN), in whom clinical, laboratory, and pathologic assessment are nondiagnostic.</td>
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<td>• Newly diagnosed MDS or MDS/MPN patients either</td>
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<td>o stratified by the IPSS or IPSS-R as intermediate risk, or</td>
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<td>o in MDS with ringed sideroblasts/RARS.</td>
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<td><strong>Limitations</strong></td>
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<td>• Repeat Genomic Sequential Analysis Panel testing is not reasonable and necessary in MDS after initial diagnosis and risk stratification.</td>
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<tr>
<td><strong>Myeloproliferative Neoplasms (MPN)</strong></td>
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<td><strong>Indications and Limitations of Coverage</strong></td>
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<td>Genomic Sequential Analysis Panel will be considered reasonable and necessary in the evaluation of blood or bone marrow samples in the following circumstances:</td>
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<td></td>
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<td></td>
<td>• Diagnosis: Clinical signs or symptoms of myeloproliferative neoplasm (MPN) or myelodysplastic/myeloproliferative overlap syndromes (MDS/MPN) when</td>
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<td></td>
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<td>o clinical, laboratory, and pathologic assessment are nondiagnostic; and</td>
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<td>o CML excluded (BCR-ABL1 negative)</td>
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<td>• Risk Stratification: Newly diagnosed PMF not already classified as high-risk by Dynamic International Prognostic Scoring System (DIPSS) Plus</td>
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<td>• Monitoring: Higher-risk MF (INT-1, INT-2, High-Risk) with progression on therapy</td>
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</table>
**Title** | **Contractor** | **ID** | **Summary**
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Genomic Sequence Analysis Panels in the Treatment of Solid Organ Neoplasms | National Government Services, Inc | L37810 | “Coverage Indications, Limitations, and/or Medical Necessity
Non-Small Cell Lung Cancer (NSCLC) Indications and Limitations of Coverage Genomic Sequential Analysis Panel will be considered reasonable and necessary in the evaluation of tumor tissue in the following clinical circumstances:
- Newly diagnosed patients with advanced (stage IIIB or IV) NSCLC, who are not treatable by resection or radiation with curative intent, and who are suitable candidates for therapy at the time of testing.
- Previously diagnosed patients with advanced (stage IIIB or IV) NSCLC, who have not responded to at least one systemic therapy, or who have progressed following resection. The patient must be a candidate for treatment at the time of the testing.
- Previously diagnosed patients with advanced (stage IIIB or IV) NSCLC, who have been resistant to at least one targeted therapy, are able to undergo tumor tissue biopsy for testing, and who are suitable candidates for additional treatment at the time of testing.
Metastatic Colorectal Cancer (mCRC) Indications and Limitations of Coverage Genomic Sequential Analysis Panel will be considered reasonable and necessary when the test is performed in a CLIA-certified laboratory qualified to perform high complexity testing, ordered by a treating physician, and the patient has:
- metastatic CRC;
- is a candidate for intensive chemotherapy with an anti-EGFR biologic agent; and
- has not had prior RAS/BRAF testing (except after initiation of anti-EGFR therapy with evidence of acquired resistance).“
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<th>Title</th>
<th>Contractor</th>
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<th>Summary</th>
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<tr>
<td>MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease</td>
<td>CGS Administrators, LLC</td>
<td>L36117</td>
<td>“This policy provides coverage for multi-gene non-NGS (Next Generation Sequencing) panel testing and NGS testing for the diagnostic workup for myeloproliferative disease (MPD), and limited coverage for single-gene testing of patients with BCR-ABL negative myeloproliferative disease (MPD). MPD includes polycythemia vera (PV), essential thrombocytopenia (ET), and primary myelofibrosis (PMF). . . . “For laboratories performing next generation sequencing (NGS or &quot;hotspot&quot;) testing platforms: Molecular testing for BCR-ABL, JAK 2, JAK, exon 12, and CALR/MPL genes by NGS is covered as medically necessary for the identification of myeloproliferative disorders.”</td>
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<td>Noridian Healthcare Solutions, LLC</td>
<td>L36180</td>
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<td>Noridian Healthcare Solutions, LLC</td>
<td>L36186</td>
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<td>Palmetto GBA</td>
<td>L36044</td>
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<td></td>
<td>Wisconsin Physicians Service Insurance Corporation</td>
<td>L36815</td>
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<tr>
<td>MolDX: Genetic Testing for Lynch Syndrome</td>
<td>CGS Administrators, LLC</td>
<td>L35349</td>
<td>“This policy limits Lynch syndrome (LS) genetic testing to a stepped approach for Microsatellite Instability and Immunohistochemistry (MSI/IHC) screening, BRAF gene mutation, MLH1 gene promoter hypermethylation and targeted mismatch repair (MMR) germ-line gene testing to all patients with colorectal cancer (CRC) and endometrial cancer regardless of age, or a multi-gene NGS or other multi-analyte methodology that is inclusive of MSI microsatellite loci, and MLH1, MSH2, MSH6 and PMS2 genes. MSI/MMR testing is also covered for adult and pediatric patients with unresectable or metastatic microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options, or colorectal cancer that has progressed following treatment with fluoropyrimidine, oxaliplatin, and irinotecan.” “For patients with unresectable or metastatic solid tumors, either MSI or IHC or a multigene NGS or other multi-analyte methodology panel inclusive of MSI microsatellite loci, and MLH1, MSH2, MSH6 and PMS2 genes is medically reasonable and necessary.”</td>
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<td>Noridian Healthcare Solutions, LLC</td>
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<td>Noridian Healthcare Solutions, LLC</td>
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<td>Palmetto GBA</td>
<td>L35024</td>
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<td></td>
<td>Wisconsin Physicians Service Insurance Corporation</td>
<td>L36793</td>
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<td>Summary</td>
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<tr>
<td>MolDX: Inivata, InVisionFirst, Liquid Biopsy for Patients with Lung Cancer</td>
<td>CGS Administrators, LLC</td>
<td>L37903</td>
<td>&quot;This policy provides limited coverage for InvisionFirstTM – Lung (Inivata, Research Triangle Park, NC) (hereafter InVision) a plasma-based, somatic comprehensive genomic profiling test (CGP) for patients with advanced (Stage IIIb/IV) non-small cell lung cancer (NSCLC):</td>
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<td>Noridian Healthcare Solutions, LLC</td>
<td>L37897</td>
<td>• At diagnosis-</td>
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<td>o When results for EGFR single nucleotide variants (SNVs) and insertions and deletions (indels); rearrangements in ALK and ROS1; and SNVs for BRAF are not available AND when tissue-based CGP is infeasible [ie, quantity not sufficient (QNS) for tissue-based CGP or invasive biopsy is medically contraindicated].</td>
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<td></td>
<td>Noridian Healthcare Solutions, LLC</td>
<td>L37899</td>
<td>or</td>
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<td>Palmetto GBA</td>
<td>L37870</td>
<td>• At progression-</td>
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<td>o For patients progressing on or after chemotherapy or immunotherapy who have not been tested for EGFR SNVs and indels; rearrangements in ALK and ROS1; and SNVs for BRAF, and for whom tissue-based CGP is infeasible;</td>
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<td>or</td>
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<td></td>
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<td>o For patients progressing on EGFR tyrosine kinase inhibitors (TKIs). If no genetic alteration is detected by InVision or if circulating tumor DNA (ctDNA) is insufficient/not detected, tissue-based genotyping should be considered.&quot;</td>
</tr>
<tr>
<td>MolDX: Minimal Residual Disease Testing for Colorectal Cancer</td>
<td>CGS Administrators, LLC</td>
<td>L38305</td>
<td>&quot;This Medicare contractor will provide limited coverage for ctDNA tests that detect minimum residual disease (MRD) in patients with a personal history of colorectal cancer. Specifically, the enclosed evidentiary review is focused on the Signatera molecular residual disease assessment test, from here on called “Signatera,” (Natera, Inc San Carlos, CA). Other tests that demonstrate equivalent analytical and clinical validity as part of a comprehensive technical assessment (TA) will similarly attain coverage for indications that are supported by the evidence and intended use within scope of this policy.</td>
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<td></td>
<td>Palmetto GBA</td>
<td>L38290</td>
<td>This Contractor provides limited coverage for MRD testing in cancer when:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1. The conditions set by NCD 90.2 are fulfilled if NGS methodology is utilized (summarized: the patient has advanced cancer; plans on being treated for said cancer, and has not been previously been tested with the same test for the same genetic content) or are not applicable (the patient does not have cancer as defined below).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. The patient has a personal history of colorectal cancer, the type and staging of which is within the intended use of the MRD test.</td>
</tr>
<tr>
<td></td>
<td>Wisconsin Physicians Service Insurance Corporation</td>
<td>L38431</td>
<td></td>
</tr>
<tr>
<td>Title</td>
<td>Contractor</td>
<td>ID</td>
<td>Summary</td>
</tr>
<tr>
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</tr>
<tr>
<td>3.</td>
<td></td>
<td></td>
<td>The identification of recurrence or progression of disease within the intended use population of the test is identified in the NCCN Guidelines as a condition that requires a definitive change in patient management.</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
<td>The test is demonstrated to identify recurrence or progression before there is clinical or radiographical evidence of recurrence or progression; and demonstrates sensitivity and specificity comparable with radiographical evidence of recurrence. For colorectal cancer, it must have a sensitivity at least equivalent to and specificity that is significantly better than serial CEA monitoring OR demonstrate equivalence with another ctDNA MRD test that has demonstrated this measuring the same analytes. Test performance must be similar to established MRD tests including Signatera.</td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
<td>The test satisfactorily completes a technical assessment that will review and confirm the analytical and clinical validity of the test. MRD testing often requires two types of assays to be performed as part of the service. First, a sample is taken from tumor diagnostic material to establish a baseline tumor signature as defined by the test methodology. This is followed by a series assays run on blood to detect the presence or recurrence of tumor based on the measured biomarkers, expression, or other analytes over various timepoints. This series of assays comprises a single test when the patient is known to have cancer. When the patient is NOT known to have cancer (specifically when there is no clinical, radiographical, or other biological evidence that tumor cells remain post treatment and subsequently the patient is no longer being subjected to therapeutic interventions for cancer), a second kind of test may exist wherein a single additional timepoint may constitute a single test.”</td>
</tr>
<tr>
<td>Title</td>
<td>Contractor</td>
<td>ID</td>
<td>Summary</td>
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<tr>
<td>------------------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>-------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| MolDX: Next-Generation Sequencing for Solid Tumors         | CGS Administrators, LLC                          | L38067 | "Criteria for Coverage
All the following must be present for coverage eligibility:
- As per NCD 90.2, this test is reasonable and necessary when:
  - the patient has either:
    - Recurrent cancer
    - Relapsed cancer
    - Refractory cancer
    - Metastatic cancer
    - Advanced cancer (stages III or IV)
  - AND has not been previously tested by the same test for the same genetic content
  - AND is seeking further treatment
- The test has satisfactorily completed a TA by MolDX for the stated indications of the test.
- The assay performed includes at least the minimum genes and genomic positions required for the identification of all FDA-approved therapies with a companion diagnostic biomarker for its intended use that can be reasonably detected by the test. Because these genes and variants will change as the literature and drug indications evolve, they are listed separately in an associated Coverage Article, as well as in the MolDX TA forms.
Situations in which Test should not be used or coverage is denied:
The test in question will be non-covered if:
- It does not fulfill all the criteria set forth in the NCD 90.2 as stated above
- Another CGP test was performed on the same tumor specimen (specimen obtained on the same date of service)
- A Technical Assessment is not completed satisfactorily by MolDX for new tests
- For tests that are currently covered but a TA submission has not been made, providers must submit complete TA materials by February 10th, 2020 or coverage will be denied" |
<p>| Noridian Healthcare Solutions, LLC                         | L38119                                          |       |                                                                                                                                          |
| Noridian Healthcare Solutions, LLC                         | L38121                                          |       |                                                                                                                                          |
| Palmetto GBA                                               | L38045                                          |       |                                                                                                                                          |
| Wisconsin Physicians Service Insurance Corporation         | L38158                                          |       |                                                                                                                                          |</p>
<table>
<thead>
<tr>
<th>Title</th>
<th>Contractor</th>
<th>ID</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>MolDX: Plasma-Based Genomic Profiling in Solid Tumors</td>
<td>CGS Administrators, LLC</td>
<td>L38065</td>
<td>&quot;Guardant360® is covered only when all of the following conditions are met:</td>
</tr>
</tbody>
</table>
|                                                                      | Palmetto GBA                                     | L38043 | • Patient has been diagnosed with a recurrent, relapsed, refractory, metastatic, or advanced solid tumor that did not originate from the central nervous system. Patients who would meet all of the indications on the FDA label for larotrectinib if they are found to have an NTRK mutation may be considered to have advanced cancer, and  
|                                                                      | Wisconsin Physicians Service Insurance Corporation | L38168 | • Patient has not previously been tested with the Guardant360® test for the same genetic content. For a patient who has been tested previously using Guardant360® for cancer, that patient may not be tested again unless there is clinical evidence that the cancer has evolved wherein testing would be performed for different genetic content. Specifically, in patients with previously tested cancer, who have evidence of new malignant growth despite response to a prior targeted therapy, that growth may be considered to be sufficiently genetically different to require additional genetic testing, and  
|                                                                      |                                                 |        | • Patient is untreated for the primary cancer being tested, or the patient is not responding to treatment (eg, progression or new lesions on treatment), and  
|                                                                      |                                                 |        | • The patient has decided to seek further cancer treatment with the following conditions:  
|                                                                      |                                                 |        |   o The patient is a candidate for further treatment with a drug that is either FDA-approved for that patient’s cancer, or has an NCCN 1 or NCCN 2A recommendation for that patient’s cancer, and  
|                                                                      |                                                 |        |   o The FDA-approved indication or NCCN recommendation is based upon information about the presence or absence of a genetic biomarker tested for in the Guardant360® assay, and  
|                                                                      |                                                 |        | • Tissue-based, CGP is infeasible (eg, quantity not sufficient for tissue-based CGP or invasive biopsy is medically contraindicated) or specifically in NSLC Tissue-based CGP has shown no actionable mutations.  
|                                                                      |                                                 |        | If no alteration is detected by Guardant360® or if ctDNA is insufficient/not detected, tissue-based genotyping should be considered. Other liquid biopsies will be covered for the same indications if they display similar performance in their intended used applications to Guardant360®."

MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies

<table>
<thead>
<tr>
<th>Title</th>
<th>Contractor</th>
<th>ID</th>
<th>Summary</th>
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</thead>
</table>
| MolDX: Next-Generation Sequencing Lab-Developed Tests for Myeloid Malignancies and Suspected Myeloid Malignancies | CGS Administrators, LLC                 | L38070 | *The following must be present FOR coverage eligibility:*  
• FOR TESTS that are specifically indicated in patients whom are known to have a MYELOID malignancy at the time of testing, NCD 90.2 applies.  
• The patient has a diagnosis of AML, MDS, or MPN. AML, MDS, and MPN are herein classified as refractory and/or metastatic cancers and fulfil the NCD 90.2 criteria.  
• The test has satisfactorily completed a TA by MolDX FOR the stated indications of the test.  
• The assay performed includes at least the minimum genes and positions indicated FOR its intended use, as described in an associated coverage Article and found in the TA FORms.  
• FOR patients that do not have a diagnosis of a MYELOID malignancy, where one is suspected, the patient must have an undefined cytopenia FOR greater than 6 months, other possible causes have been reasonably excluded.  
• Testing is performed on bone marrow biopsies or peripheral blood samples.  
Situations in which Test should not be used or coverage is denied:  
The test in question will be non-covered if:  
• A Technical Assessment has not been satisfactorily completed by MolDX. FOR TESTS that are currently covered but a TA submission has not been made, providers must submit complete TA materials by February 10th, 2020 or coverage will be denied.  
• Another NGS test was performed on the same surgical specimen/ blood draw (specimen obtained on the same date of service).  
• Testing falls within scope of NCD 90.2 and has been tested with the same test FOR the same genetic content.* |
<p>|                                                                      | Noridian Healthcare Solutions, LLC      | L38123 |                                                                                                                                                                                                     |
|                                                                      | Noridian Healthcare Solutions, LLC      | L38125 |                                                                                                                                                                                                     |
|                                                                      | Palmetto GBA                            | L38047 |                                                                                                                                                                                                     |
|                                                                      | Wisconsin Physicians Service Insurance Corporation | L38176 |                                                                                                                                                                                                     |</p>
<table>
<thead>
<tr>
<th>Title</th>
<th>Contractor</th>
<th>ID</th>
<th>Summary</th>
</tr>
</thead>
</table>
| MolDX: Minimal Residual Disease Testing for Colorectal Cancer | Palmetto GBA                   | L38290| "This Medicare contractor will provide limited coverage for ctDNA tests that detect minimum residual disease (MRD) in patients with a personal history of colorectal cancer."
                                          |       |       | (Signatera molecular residual disease assessment test and equivalent tests)                                                                                                                                 |
| Molecular Pathology Procedures             | National Government Services, Inc | L35000| "Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (EG, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGfra, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed is considered not medically necessary except when used to guide treatment decision making in individuals with non-small cell lung cancer (please refer to LCD L36376)." |

Abbreviations: CEA, carcinoembryonic antigen; CLIA, Clinical Laboratory Improvement Amendments; CML, chronic myeloid leukemia; ctDNA, circulating tumor DNA; IPSS, International Prognostic Scoring System; IPSS-R, IPSS-revised; LCD, local coverage determination; MolDx, molecular diagnostics; NCCN, National Comprehensive Cancer Network; NCD, national coverage determination; PMF, primary myelofibrosis; RARS, refractory anemia with ringed sideroblasts.
Appendix B: Guiding Questions for Stakeholders

As specified in PCORI’s Statement of Work, ECRI conducted interviews with a range of stakeholders, including health care providers (2 clinical oncologists), payers (2 representatives of commercial payers, one from a nonprofit health system and one from the Centers for Medicare & Medicaid Services), patients (one current and one former patient), and representatives from patient advocacy groups (2 representatives from unique organizations supporting patients with cancer). ECRI conducted 12 calls with stakeholders, including 2 with each clinical expert. Average call duration was 1 hour. Below are lists of questions for each stakeholder group that the ECRI team used to guide discussions. PCORI approved the questions before we scheduled calls. These questions were meant to guide discussion, and not all questions for a given stakeholder group were addressed on every call.

Clinical Experts

Technical questions (project scope/content):

- Should we distinguish studies that look at patient populations with established molecularly directed therapies (eg, first-line treatment of NSCLC for which there are multiple FDA-approved therapies) from studies of patient populations with no established molecularly directed therapies?
- Should we include for analysis studies reporting only on actionable mutations?
- Is BRCA1/BRCA2 testing a panel just because it is testing 2 genes?
- Should we assess studies of panels vs whole-exome sequencing, whole-genome sequencing, and RNA sequencing (assessing sequence variants, not just for expression analysis)? Are all these in scope, or should we restrict our evidence to multigene panels?
- Should assessment of tumor mutational burden (typically used for PD-1/PD-L1 inhibitors) be included in our scope?
- How is clinical outcomes data reported on only tens of patients considered (eg, trial enrolled 200 patients, treatment change to targeted therapy in 50 patients, and overall survival/progression-free survival reported only for 10 patients [10/50] who received therapy)?

Questions for broader discussion:

- Do you use NGS panel tests for your patients? Why or why not? If so, what panel tests have you ordered?
- What factors influence your decision to order genetic testing through a particular lab?
- Do you or your organization have a designated laboratory for particular tests, or are physicians free to select based on personal preference?
- Do you or your organization have a protocol for evaluating and introducing new tests into your clinical practice?
- Are your patients receptive when presented with the option for genetic testing?
- Do you discuss potential out-of-pocket costs with patients before ordering tests?
- How does a patient’s insurance factor into your decision to (1) order a genetic test and (2) select a laboratory for testing?
- How often does a patient’s insurance company deny a claim for a genetic test? Are you involved in disputes (eg, peer-to-peer calls)?
- Does your organization conduct any in-house genetic/genomic testing?
• For what patients/situations are you more likely to use NGS panel tests? Approximately what percentage of your patients with cancer are offered NGS panel testing?
• For what types of cancer are NGS tests more frequently ordered?
• What factors dictate the timing of ordering a genetic test (eg, stage of cancer, previous failed therapies, guideline dictated)?
• How important is the availability of direct clinical utility data for an NGS panel? Or does evidence that the test works (availability of clinical and analytic validity data) and covers the desired variants suffice?
• If multiple NGS panel tests are available, what factors decide which test is chosen (eg, clinical utility data, insurance coverage, number of variants covered/panel size)?
• How much weight do the results from an NGS test have on treatment decisions or changes? If an appropriate targeted therapy is available, would patients be immediately switched?
• Is there any preference among clinicians for FDA-approved tests (if available) over laboratory-developed tests?
• Is there any preference among clinicians for using NGS panel tests over single-gene tests, or vice versa? Discuss reasons.
• Do you sometimes combine somatic testing with germline testing to inform treatment decisions?
• In your practice, do genetic counselors have a role in patient engagement regarding NGS results that inform treatments?
• Are the summary reports of NGS test results understandable, given the time constraints you have in practice?
• How extensively do you use the results from NGS multigene panel tests? What information is most useful? Is any information provided that you do not find useful?
• Does use of NGS panel tests engender more confidence in your treatment decisions?
• What information from NGS tests do you communicate to patients? Are they able to understand the results you present to them? Are other staff (eg, genetic counselors, nurses) available to assist with communicating test results?
• Do you evaluate the evidence supporting use of NGS panel tests to guide treatment of patients with cancer? How often do you survey the literature, and how extensive is your review?
• What additional studies do you think are needed to gauge genomic sequencing’s impact on cancer treatment?
• How do you handle data on variants of uncertain significance (VUSs)? Do they concern you? Do you find them confusing? What, if anything, about these data do you communicate to patients? Is this helpful or confusing to them?
• Would you find more guidance on VUSs to be helpful in interpreting results or communicating with patients? Why or why not?
• What is your opinion on expansive tests that include genes/variants without known disease association—do you welcome or oppose the blurring of the lines between clinical care and research? Do you have any qualms ordering such tests and indicating that they are medically necessary?
• What concerns do you have with covering NGS gene panels as opposed to single-gene tests or smaller multigene panels?
• What evidence do you require for coverage of NGS panel tests? Do you require data on clinical utility? What outcome measures do you accept as evidence?
• Ideally, what study designs would you like to see for clinical utility data?
• Does your organization have an upper limit on the number of genes a panel can include to remain reimbursable?
• How strongly are your coverage decisions based on clinical practice guideline recommendations vs clinical studies? For instance, if published guidelines support use of a test that is not supported by clinical utility data, are you likely to cover it?
• Does your organization have specific criteria for covering NGS panel tests? How much does the cost factor into your coverage decisions?
• How do factors such as patient “convenience” (liquid vs tissue biopsy) and avoidance of potential ancillary costs from adverse events (such as those related to invasive procedures) influence your coverage decisions?
• Do particular professional organizations or other payers (eg, CMS) strongly influence your coverage decisions?
• What is the importance of FDA approval for an NGS test in the decision to provide coverage?
• Based on NGS test results, if off-label targeted therapies are available for patients, would they be covered?
• What importance do you give to cost-effectiveness studies in coverage decisions?

Patients
• Please describe your experience with the use of genomic testing for treatment of cancer. Was this personal experience?
• How did you find out about genetic testing as an option?
• Were you consulted before the test was ordered?
• Were you offered genetic counseling before taking the test?
• What panel test did you take?
• Did you understand the purpose of genetic testing? What expectations did you have about this technology?
• Who communicated your test results to you?
• Were you able to understand the results? What did you not understand or find confusing?
• Did the test results change your treatment recommendations in any way? If so, how? Did your physician recommend a more or less aggressive treatment plan?
• (For germline tests) how was your decision to pursue/avoid testing influenced by family members? How did having genetic testing make you feel about your physician’s recommended treatment or your assessment of treatment options? Would you recommend this testing to other patients?
• Did your health insurance cover the cost of the test? If not, did you pay out of pocket, and did the test developer/provide you with a discount?
Patient Advocacy Representatives

- What are your or your organization’s views on the use of genomic sequencing for managing patients with cancer?
- Are most patients aware of this technology, and do they understand its intended purpose?
- In what percentage of cases do you think genetic testing is providing enough information to make a treatment decision in managing a patient’s cancer?
- Do you have any concerns with the widespread use of this technology?
- Are you aware of disparities in access to care based on socioeconomic factors? Do you have any programs in place to assist patients with the cost of testing/treatment?
Appendix C: Literature Search Methods

From June 2020 through November 2020, we conducted bibliographic database searches for peer reviewed journal literature. We also performed gray literature searches for clinical trials, coverage policies, regulatory information, and trends relating to genomic sequencing for guiding cancer management.

Bibliographic Database Searches (EMBASE.com and Google Scholar):

We performed our bibliographic searches in EMBASE.com (which searches MEDLINE and EMBASE together). Search results were limited to studies published between 2010 and 2020 and added to the database on or before August 28, 2020.

We developed 3 specific, peer reviewed search strategies based on the stated project goals and guiding questions and under the guidance of ECRI analysts. We present these strategies in the table below in EMBASE.com syntax (using EMTREE controlled vocabulary and free-text terms).

We also searched Google Scholar to identify journal articles that named a commercial next-generation sequencing test in the full text of a publication. This approach was designed to pick up studies not identified from EMBASE.com searches, since this platform does not search full-text articles. The Google Scholar search strategies included commercial test names along with key terms of interest (eg, clinical utility, clinical validity, decision making, overall survival, progression-free survival, treatment decision). All unique publications identified via Google Scholar were later retrieved via a title search in EMBASE.com.

Table C-1. Bibliographic Search Strategy Performed in EMBASE.com

<table>
<thead>
<tr>
<th>Set No.</th>
<th>Concept</th>
<th>Search statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Search 1: Studies involving patient testing and focusing on the following areas of interest: treatment outcomes, clinical utility or decision making, adverse events or harms, clinical validity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#1</td>
<td>Cancer (EMTREE controlled vocabulary)</td>
<td>‘malignant neoplasm'/exp/mj OR metastasis/exp/mj</td>
</tr>
<tr>
<td>#2</td>
<td>Cancer (descriptive text words)</td>
<td>(adenocarcinoma* OR adenoma* OR cancer* OR carcinoma* OR leukaemia* OR leukemia* OR lymphoma* OR malignan* OR melanoma* OR metastas* OR metastat* OR neoplas* OR oncolog* OR sarcoma* OR tumor* OR tumour*):ti</td>
</tr>
<tr>
<td>#3</td>
<td>Combine cancer sets</td>
<td>#1 OR #2</td>
</tr>
<tr>
<td>#4</td>
<td>Next-generation sequencing (NGS; EMTREE controlled vocabulary)</td>
<td>‘high throughput sequencing’/exp/mj</td>
</tr>
<tr>
<td>Set No.</td>
<td>Concept</td>
<td>Search statement</td>
</tr>
<tr>
<td>--------</td>
<td>---------</td>
<td>------------------</td>
</tr>
<tr>
<td>#5</td>
<td>NGS (descriptive text words)</td>
<td>('high throughput' OR NGS OR sequencing):ti OR ('AMP-NGS' OR 'amplicon capture' OR 'amplicon sequencing' OR ((deep OR ultradeep) NEXT/2 sequencing) OR DNA-seq' OR DNAseq OR 'exom* sequencing' OR ('genomically-matched' AND test*) OR 'genomic profiling' OR 'genom* sequencing' OR ('high throughput' NEXT/2 sequencing) OR 'hybrid* capture' OR ('massive* parallel' NEXT/2 sequencing) OR 'miRNA-seq' OR miRNAseq OR 'miRNA sequencing' OR 'molecular profiling' OR ('next-gen' OR nextgen OR 'next generation') NEXT/2 sequencing) OR (panel NEAR/5 sequencing) OR pyrosequencing OR 'RNA-seq' OR RNAseq OR 'RNA sequencing' OR 'single molecule real-time sequencing' OR 'SMRT sequencing' OR 'target enrichment' OR (target* NEXT/2 sequencing) OR 'transcriptome sequencing');ti,ab</td>
</tr>
<tr>
<td>#6</td>
<td>Platforms for performing NGS</td>
<td>(454 OR ampiliseq* OR archerdx OR fusionplex* OR genereader* OR haloplex* OR hiseq* OR illumina OR 'ion PGM' OR 'ion proton' OR 'ion torrent' OR miniseq* OR miseq* OR nextseq* OR nimblegen* OR novaseq* OR 'omics core*' OR omicsoft OR pacbio OR qiagen OR qiaseq* OR raindance* OR seqcap* OR solexa* OR sureselect OR thermofisher OR 'thermo fisher' OR thunderbolts* OR thunderstorm*:ti,ab,dn AND sequencing:ti,ab</td>
</tr>
<tr>
<td>#7</td>
<td>Combine EMTREE, descriptive, and platform NGS sets</td>
<td>#4 OR #5 OR #6</td>
</tr>
<tr>
<td>#8</td>
<td>Limit NGS set to studies likely to involve clinical testing</td>
<td>#7 AND ('genetic screening'/exp OR (commercial* OR companion OR panel OR personal* OR precision OR 'real world');ti,ab OR ((clinical OR genetic OR genomic OR molecular OR mutational OR NGS OR 'next generation' OR pharmacogenetic OR pharmacogenomic OR profiling OR 'tumor profiling' OR 'tumor profiling') NEXT/2 (assay* OR test*)):ti,ab OR test*:ti)</td>
</tr>
<tr>
<td>#9</td>
<td>Treatment outcomes (area of interest)</td>
<td>'quality of life'/exp OR survival/exp OR 'treatment outcome'/exp OR 'treatment response'/de OR (outcome* OR respon*);ti OR (death* OR morbidity OR mortality OR (patient NEXT/1 outcome*) OR QOL OR 'quality of life' OR (rate NEAR/1 respon*) OR survival OR ((therap* OR treatment*) NEAR/2 (monitor OR outcome* OR respon*)));ti,ab</td>
</tr>
<tr>
<td>#10</td>
<td>Clinical utility or decision making (area of interest)</td>
<td>'clinical practice'/de OR 'decision making'/exp OR (clinical NEXT/1 (application* OR benefit* OR decision* OR effectiveness OR impact* OR implication* OR management OR outcome* OR practice OR setting* OR use OR utility));ti,ab OR 'decision making':ti,ab OR (inform* AND (therap* OR treat*)):ti,ab OR 'personal utility':ti,ab OR (actionable OR advantage* OR benefit* OR decid* OR decision* OR efficac* OR ((guid* OR select*) AND (therap* OR treat*))) OR inform OR informing OR role OR practice OR targeted OR use OR usefull* OR utility OR valu*);ti</td>
</tr>
<tr>
<td>#11</td>
<td>Adverse events or harms (area of interest)</td>
<td>'adverse event'/exp OR ((adverse NEXT/1 (effect* OR event* OR reaction*));ti,ab</td>
</tr>
<tr>
<td>#12</td>
<td>Clinical validity (area of interest)</td>
<td>'diagnostic error'/exp OR 'predictive validity'/de OR 'predictive value'/de OR 'sensitivity and specificity'/de OR ((clinical* NEXT/1 valid*) OR concordance OR (false NEXT/1 (positive* OR negative*))) OR 'likelihood function* OR 'likelihood ratio* OR 'predictive validity' OR 'predictive value' OR sensitiv* OR specific OR specificity);ti,ab</td>
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<tr>
<td>#13</td>
<td>Combine areas of interest</td>
<td>#9 OR #10 OR #11 OR #12</td>
</tr>
<tr>
<td>Set No.</td>
<td>Concept</td>
<td>Search statement</td>
</tr>
<tr>
<td>--------</td>
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<td>------------------</td>
</tr>
<tr>
<td>#14</td>
<td>Combine cancer, NGS, and areas of interest sets</td>
<td>#3 AND #8 AND #13</td>
</tr>
<tr>
<td>#15</td>
<td>Apply general hedges</td>
<td>See General hedges at the end of this table</td>
</tr>
</tbody>
</table>

**Search 2: Studies comparing NGS-guided cancer treatment vs non-NGS guided cancer treatment (eg, physician-choice, single-gene or other alternative testing, standard treatment)**

<p>| #1     | Cancer (EMTREE controlled vocabulary) | 'malignant neoplasm'/exp/mj OR metastasis/exp/mj |
| #2     | Cancer (descriptive text words) | (adenocarcinoma* OR adenoma* OR cancer* OR carcinoma* OR leukaemia* OR leukaemia* OR lymphoma* OR malignan* OR melanoma* OR metastas* OR metastat* OR neoplas* OR oncolog* OR sarcoma* OR tumor* OR tumour*):ti |
| #3     | Combine cancer sets | #1 OR #2 |
| #4     | NGS (EMTREE controlled vocabulary) | 'high throughput sequencing'/exp/mj |
| #5     | NGS (descriptive text words) | ('high throughput’ OR NGS OR sequencing):ti OR ('AMP-NGS’ OR ‘amplicon capture’ OR ‘amplicon sequencing’ OR ((deep OR ultradeep) NEXT/2 sequencing) OR ‘DNA-seq’ OR DNAseq OR ‘exom’ sequencing’ OR (‘genomically-matched’ AND test*) OR ‘genomic profiling’ OR ‘genom* sequencing’ OR (‘high throughput’ NEXT/2 sequencing) OR ‘hybrid* capture’ OR (‘massive* parallel’ NEXT/2 sequencing) OR ‘mirNA-seq’ OR miRNAseq OR ‘miRNA sequencing’ OR ‘molecular profiling’ OR ((‘next-gen’ OR nextgen OR ‘next generation’) NEXT/2 sequencing) OR (panel NEAR/5 sequencing) OR pyrosequencing OR ‘RNA-seq’ OR RNAseq OR ‘RNA sequencing’ OR ‘single molecule real-time sequencing’ OR ‘SMART sequencing’ OR ‘target enrichment’ OR (target* NEXT/2 sequencing) OR (‘transcriptome sequencing’):ti,ab |
| #6     | Platforms for performing NGS | (454 OR ampliseq* OR archerdx OR fusionplex* OR genereader* OR haloplex* OR hiseq* OR illumina OR ‘ion PGM’ OR ‘ion proton’ OR ‘ion torrent’ OR miniseq* OR miseq* OR nextseq* OR nimblegen* OR novaseq* OR ‘omics core’* OR oncomine* OR pacbio OR qiagen OR qiaseq* OR raindance* OR seqcap* OR solexa* OR sureselect* OR thermofisher OR ‘thermo fisher’ OR thunderbolts* OR thunderstorm*):ti,ab,dn AND sequencing:ti,ab |
| #7     | Combine EMTREE, descriptive, and platform NGS sets | #4 OR #5 OR #6 |
| #8     | Combine cancer and NGS sets | #3 AND #7 |
| #9     | Limit to meta-analyses and systematic reviews | #8 AND (‘meta analysis’/de OR ‘randomized controlled trial (topic)’/de OR ‘systematic review’/de OR (EMBASE OR ‘meta analysis’ OR ‘meta analytic’ OR metaanaly* OR RCTs OR ‘research synthesis’ OR scopus* OR (systematic NEXT/3 review)):ti,ab OR (‘critical review’ OR ‘evidence based’ OR ‘pooled analysis’):ti OR [cochrane review]/lim) |</p>
<table>
<thead>
<tr>
<th>Set No.</th>
<th>Concept</th>
<th>Search statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>#10</td>
<td>Limit to comparative studies (randomized and nonrandomized)</td>
<td>#8 AND (‘random sample’/de OR ‘randomized controlled trial’/de OR randomization/de OR (random* OR RCT):ti,ab OR ‘comparative study’/exp OR ‘controlled study’/exp OR (‘between groups’ OR ‘case control’* OR compar* OR ‘control group’* OR ‘controlled study’ OR ‘controlled trial’ OR ‘cross-over’ OR crossover OR ‘double blind’ OR ‘double blinded’ OR ‘matched controls’):ti,ab OR (versus OR vs):ti)</td>
</tr>
<tr>
<td>#11</td>
<td>Combine study sets</td>
<td>#9 OR #10</td>
</tr>
<tr>
<td>#12</td>
<td>Limit to studies likely to focus on NGS</td>
<td>#11 AND (‘molecular marker’* OR ‘mutation’* test’* OR NGS OR panel OR panels OR personal* OR precision OR profiling OR sequencing OR WES OR WGS OR ‘whole exome’ OR ‘whole genome’):ti</td>
</tr>
<tr>
<td>#13</td>
<td>Limit to studies likely to involve comparisons</td>
<td>#12 AND (compar* OR control* OR random* OR versus OR vs):ti</td>
</tr>
<tr>
<td>#14</td>
<td>Limit to studies likely to involve alternatives to NGS-guided treatment</td>
<td>#12 AND (‘best supportive’ OR ((conventional OR standard OR usual) NEXT/2 (care OR chemotherap* OR therap* OR treatment*)) OR (physician NEXT/1 (choice OR directed OR guided OR selected)) OR ‘sequential test’* OR ‘single-gene’):ti,ab OR (conventional OR standard OR usual):ti)</td>
</tr>
<tr>
<td>#15</td>
<td>Combine sets</td>
<td>#13 OR #14</td>
</tr>
<tr>
<td>#16</td>
<td>Apply general hedges</td>
<td>See General hedges at the end of this table</td>
</tr>
</tbody>
</table>

**Search 3: Studies involving commercial NGS tests**

<p>| #1     | Cancer (EMTREE controlled vocabulary) | ‘malignant neoplasm’/exp/mj OR metastasis/exp/mj |
| #2     | Cancer (descriptive text words) | (adenocarcinoma* OR adenoma* OR cancer* OR carcinoma* OR leukaemia* OR leukemia* OR lymphoma* OR malignant* OR melanoma* OR metastas* OR metastat* OR neoplas* OR oncolog* OR sarcoma* OR tumor* OR tumour*):ti |
| #3     | Combine cancer sets | #1 OR #2 |
| #4     | FDA companion diagnostics and other cleared or approved NGS tests | (clonoseq* OR FD1CDx* OR ‘foundation cdx’* OR foundationcdx* OR ‘foundation focus’* OR foundationfocus* OR ‘foundation one’* OR foundationone* OR guardant* OR ‘msk impact’* OR mskimpact* OR mychoice* OR ‘omics core’* OR (oncomine* AND panel) OR (praxis* AND RAS)):ti,ab,dn |</p>
<table>
<thead>
<tr>
<th>Set No.</th>
<th>Concept</th>
<th>Search statement</th>
</tr>
</thead>
<tbody>
<tr>
<td>#5</td>
<td>Other commercial NGS tests</td>
<td>‘AML molecular profile’ OR (amplicon* AND panel) OR cancercomplete* OR ‘cancer plex*’ OR cancerplex* OR cancerselect* OR clarifind* OR clearid* OR ‘CNS tumor gene set*’ OR ‘decisiondx-CMSeq*’ OR ‘decisiondx-UMSeq*’ OR ‘edgeseq ALKPlus*’ OR ‘EXaCT-1*’ OR ‘fusion plex*’ OR fusionplex* OR ‘GEM cancer panel*’ OR genekey* OR ((‘gene-read*’ OR generead*) AND panel) OR genetrails* OR glioseq* OR ‘GPS cancer*’ OR haloplex* OR inversionfirst* OR inversionseq* OR liquiddx* OR ‘lung targeted profile*’ OR ‘lymphoid molecular profile*’ OR ‘mate pair*’ OR matepair* OR ‘melanoma targeted profile*’ OR ‘MPN extended reflex*’ OR ‘MPN targeted profile*’ OR ‘msk-access*’ OR mskaccess* OR ‘myeloid molecular profile*’ OR-novofocus* OR ‘onco carta*’ OR oncocarta* OR omniseq* OR oncoxone* OR oncogxselect* OR ‘oncology hotspot panel*’ OR oncoseq* OR oncopanel* OR oncovantage* OR ‘P.A.R.I.S.’ OR ‘PARPi CDx’ OR PCDx* OR ‘pan-cancer mutation panel*’ OR ‘PGxOnco*’ OR ‘PGxOne*’ OR ‘plasmaselect*’ OR ‘prima comprehensive*’ OR ‘prima liquid biopsy*’ OR ‘prima somatic cancer mutation*’ OR ‘signatera*’ OR ‘solid tumor mutation panel*’ OR ‘tempus xE*’ OR ‘tempus xf*’ OR ‘tempus xt*’ OR (tempus AND (assay OR panel OR sequencing)) OR (trailblaze* AND Pharos*) OR trovera* OR ((‘tru-seq*’ OR trueq*) NEXT/4 panel) OR ((‘true sight*’ OR trusight*) AND panel) OR tumormext* OR ‘whole genome transcriptome sequencing*’ OR WGTS*:ti,ab,dp</td>
</tr>
<tr>
<td>#6</td>
<td>Combine commercial NGS sets</td>
<td>#4 OR #5</td>
</tr>
<tr>
<td>#7</td>
<td>Combine cancer and commercial NGS sets</td>
<td>#3 AND #6</td>
</tr>
<tr>
<td>#8</td>
<td>Apply general hedges</td>
<td>See General hedges at the end of this table</td>
</tr>
</tbody>
</table>

**General hedges applied to each search**

| Exclude animal and experimental studies | NOT (((animals)/lim NOT (humans)/lim) OR (animal* OR experimental OR (vitro NOT vivo) OR canine OR dog OR dogs OR mouse OR mice OR rabbit* OR rat OR rats OR rodent* OR sheep OR swine):ti) |
| Limit to English-language publications and results with abstracts | AND [english]/lim AND [abstracts]/lim |
| Remove undesired publication and study types (eg, case reports, conferences, editorials) | NOT (‘conference paper’/exp OR [conference abstract]/lim OR [conference paper]/lim OR [conference review]/lim OR ‘case report’ OR book OR editorial OR erratum OR letter OR note OR ‘short survey’/de OR (book OR conference OR editorial OR erratum OR note OR ‘short survey’)):ti OR (‘a case’ OR ‘year old’):ti,ab OR (book OR ‘conference proceeding’):pt OR (‘case report’ OR comment OR letter):ti |
| Limit to results published since 2010 | AND [2010-2020]/py |
| Limit to results added to EMBASE on or before August 28, 2020 | NOT [29-8-2020]/sd |
Clinical Trials and NIH Funding Announcements

We searched ClinicalTrials.gov and the PCORI website (www.pcori.org) with 2 aims: (1) to identify PCORI-funded trials in this topic area and (2) to identify premarket and postmarket ongoing trials. Our search included the following terms and concepts: patient-centered research outcomes institute; PCORI; various cancer terms; various descriptive NGS terms; various commercial NGS tests; phase III/3; phase IV/4; industry funded; pivotal; expanded access; off-label.

ECRI Genetic Test Assessment Database

We searched the ECRI Genetic Test Assessment database of genetic test profiles to confirm that high profile, commercially available tests were captured by our structured searches, and to potentially identify additional commercially available tests. As many of these tests are regulated through CLIA, they are not identifiable through FDA.

Google Search Engine

We searched Google for background information and trends on NGS testing for guiding cancer management. Our strategy included the following search terms and concepts: genome sequencing; genomic sequencing; next-generation sequencing; targeted therapy; coverage; framework; policy; emerging; trends.

PCORI Health Care Horizon Scanning System (in-house leads database)

We searched our in-house database for news pertaining to genomic sequencing and announcements of tests and targeted therapies. We scanned our topics, trends, and leads areas for various descriptive NGS terms.

Third-Party Payer Coverage Policies

We searched the websites of 9 third-party payers representing geographic areas across the United States, by descriptive NGS terms as well as commercial test names.

US Centers for Medicare & Medicaid Services Website

We searched the CMS website for national coverage policies and supporting documents, local coverage articles, local coverage determinations, and payment models. Our search strategies included descriptive NGS terms as well as commercial test names.

US Food and Drug Administration Website

FDA’s List of Cleared or Approved Companion Diagnostic Devices was our starting point in compiling names of genomic sequencing tests for guiding cancer management (ie, selecting targeted therapy). We also used FDA’s 510(k) premarket notification and premarket approval databases to gather names of noncompanion, FDA-regulated genomic sequencing tests. We checked all lists at regular intervals for newly cleared/approved tests. Finally, we searched FDA guidance and related materials for information on regulatory processes for NGS tests.
Appendix D: Literature Review Methodology

Literature Screening Process

We uploaded all literature retrieved by our structured searches to a literature management system (Distiller SR, Evidence Partners, Ottawa, Ontario, Canada) for reference tracking and review. Literature screening proceeded in 3 broad levels: (1) title and abstract screening; (2) full-text screening; and (3) re-review and final selection of full-text studies for inclusion.

In the title/abstract screening stage (level 1 screening), individual analysts reviewed only the titles or titles and abstracts of published articles and gray literature. Any literature obviously outside the project scope was excluded based on evaluation of the article title. For references with titles indicating potential relevance to our literature review, analysts reviewed the abstracts to render a decision. (Note that we did not include a title-only screening phase as is typically done because preliminary searches were well focused and did not retrieve many significantly off-topic studies.) Any references that did not contain an abstract (eg, gray literature sources) were judged for inclusion to level 2 screening based on their titles. Inclusion of these latter references for further review was liberal to avoid excluding potentially relevant literature. However, for studies reporting only on detected mutations as an outcome, we required that the abstract report the term *actionable mutation* or some other equivalent description. We imposed this requirement because the number of studies reporting solely on mutations discovered by genomic testing was very large and collecting all such studies for full-text review would have rendered the screening process infeasible in the project’s allotted time. During level 1 screening, analysts specified in Distiller whether studies were excluded based on title-only or title-and-abstract reviews.

At the outset, to ensure consistency in screening decisions between analysts, we selected a subset of roughly 10% of references to be screened by 2 or more analysts (double-screening). Conflicts among analysts were discussed and resolved. After this stage, individual analysts screened the remaining titles and abstracts. Given the substantial literature base and limited time to complete this project, limited double-screening was a more efficient method by which to progress to the crucial full-text review stage. Most references were easily decided upon based on the inclusion criteria outlined above. For title and abstract screening, we maintained a conservative approach by forwarding any difficult decisions to the next level of screening.

As title/abstract review proceeded, electronic copies of full-text publications of studies included for level 2, full-text screening were acquired and uploaded to Distiller. Once level 1 screening was complete, analysts reviewed full-text studies as they arrived. Distiller review forms were created to abstract basic data, such as the study’s type and design, the subject matter reported on, the name of the tests used, the relevant outcomes reported, and the number of study participants. Studies excluded after full-text review were also tracked along with brief reasons for their exclusion.

Once level 2 screening was complete, analysts were assigned their individual sections of the report, and final screening, level 3, commenced. Analysts reviewed again the full-text studies specific to their section and made final decisions on inclusion in the report.

We also reviewed retrieved gray literature, governmental and private-payer policies, clinical trial records, selected clinical practice guidelines, and perspectives papers for additional contextual information. Due to the large volume of literature on this subject, we included only peer reviewed published studies for our evidence map summaries.
Data Extraction and Analysis

As noted above, first-level data abstraction took place during level 2 full-text screening, and we recorded basic information in Distiller. We constructed evidence maps in Microsoft Excel using spreadsheet reports generated from Distiller that contained basic study information (e.g., author, title, publication year) and the abstracted data. We used spreadsheet sorting to generate counts of different groups of studies (e.g., single-arm observational). We recorded second-level data abstraction (e.g., additional outcomes reported in the study or values reported for concordance) in Excel spreadsheets to prepare to construct evidence maps.

We did not provide formal quality assessments (risk of bias analysis) for individual studies or overall strength of evidence ratings for various outcomes. This level of analysis was beyond the scope of this report. However, we comment narratively on the overall quality and limitations of the most widely encountered study types in the literature, particularly comparative and single-arm observational studies, as these composed, respectively, the most important and most voluminous literature on this topic.
Appendix E: Literature Flow Diagram of Study Inclusion/Exclusion

Figure E-1. Literature Flow Diagram

4913 citations identified by searches

3968 citations excluded at title/abstract level
Citations were off topic, did not address a question of interest, or did not report an outcome of interest.

945 full-text studies reviewed

624 articles excluded after 2 rounds of full-text review
Reasons for exclusion:
• Narrative reviews
• Not a study (e.g., protocol or opinion)
• Did not address a guiding question
• No outcomes of interest
• Test not offered in US or not CLIA certified
• Research use only test
• Other reasons

321 included studies

Abbreviations: CLIA, Clinical Laboratory Improvement Amendments
## Appendix F: List of NGS Tests Used in Studies

### Table F-1. Commercial Next Generation Sequencing (NGS) Tests Included in Literature Review

<table>
<thead>
<tr>
<th>NGS panel test</th>
<th>Test developer, location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Archer™ FusinoPlex™ Solid Tumor Panel</td>
<td>Invitae Corp, Boulder, Colorado, USA</td>
</tr>
<tr>
<td>CancerPlex®</td>
<td>KEW, Inc, Waltham, Massachusetts, USA</td>
</tr>
<tr>
<td>Caris</td>
<td>Caris® Life Sciences, Inc, Irving, Texas, USA</td>
</tr>
<tr>
<td>clonoSEQ®</td>
<td>Adaptive Biotechnologies Corp, Seattle, Washington, USA</td>
</tr>
<tr>
<td>Columbia Combined Cancer Panel</td>
<td>Personalized Genome Medicine, Department of Pathology and Cell Biology, Columbia University, New York, New York, USA</td>
</tr>
<tr>
<td>FoundationOne</td>
<td></td>
</tr>
<tr>
<td>FoundationOne® CDx</td>
<td>Foundation Medicine, Inc, Cambridge, Massachusetts, USA</td>
</tr>
<tr>
<td>FoundationOne® Heme</td>
<td></td>
</tr>
<tr>
<td>Foundation One® Liquid CDx</td>
<td></td>
</tr>
<tr>
<td>GeneTrails®</td>
<td>Knight Diagnostic Laboratories, Oregon Health &amp; Science University, Portland, Oregon, USA</td>
</tr>
<tr>
<td>Geneseeq</td>
<td>Geneseeq Technology, Inc, Toronto, Ontario, Canada</td>
</tr>
<tr>
<td>GlioSeq®</td>
<td>Molecular &amp; Genomic Pathology Laboratory, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA</td>
</tr>
<tr>
<td>GPS Cancer™</td>
<td>NantHealth, Inc, El Segundo, California, USA</td>
</tr>
<tr>
<td>Guardant360®</td>
<td>Guardant Health, Inc, Redwood City, California, USA</td>
</tr>
<tr>
<td>xGen® Pan-Cancer Panel</td>
<td>Integrated DNA Technologies, Inc, Coralville, Iowa, USA</td>
</tr>
<tr>
<td>InVisionFirst®-Lung</td>
<td>Inivata, Inc, Research Triangle Park, North Carolina, USA</td>
</tr>
<tr>
<td>InVisionSeq™</td>
<td>Inivata, Inc, Research Triangle Park, North Carolina, USA</td>
</tr>
<tr>
<td>JAX ActionSeq™</td>
<td>The Jackson Laboratory, Bar Harbor, Maine, USA</td>
</tr>
<tr>
<td>JAX Cancer Treatment Profile™</td>
<td>The Jackson Laboratory, Bar Harbor, Maine, USA</td>
</tr>
<tr>
<td>Mayo Clinic 50-Gene Panel (Solid Tumor-Targeted Cancer Gene Panel, Next-Generation Sequencing)</td>
<td>Mayo Clinic Laboratories, Rochester, Minnesota, USA</td>
</tr>
<tr>
<td>myChoice® CDx</td>
<td>Myriad Genetics, Inc, Salt Lake City, Utah, USA</td>
</tr>
<tr>
<td>Omniseq Comprehensive®</td>
<td>Omniseq, Inc, Buffalo, New York, USA</td>
</tr>
<tr>
<td>Oncomine™ Dx</td>
<td>Thermo Fisher Scientific, Inc, Waltham, Massachusetts, USA</td>
</tr>
<tr>
<td>OncoPanel</td>
<td>Center for Advanced Molecular Diagnostics, Department of Pathology at Brigham and Women’s Hospital, Boston, Massachusetts, USA</td>
</tr>
<tr>
<td>NGS panel test</td>
<td>Test developer, location</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>OncoPlex</td>
<td>University of Washington, Department of Laboratory Medicine, Seattle, Washington, USA</td>
</tr>
<tr>
<td>OncoSeq</td>
<td>University of Pittsburgh Medical Center, Molecular &amp; Genomic Pathology Laboratory, Pittsburgh, Pennsylvania, USA</td>
</tr>
<tr>
<td>OncoVantage®</td>
<td>Quest Diagnostics, Inc, Secaucus, New Jersey, USA</td>
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<td>PCDx™</td>
<td>Paradigm Diagnostics, Inc, Phoenix, Arizona, USA</td>
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<tr>
<td>PGDx</td>
<td>Personal Genome Diagnostics, Inc, Baltimore, Maryland, USA</td>
</tr>
<tr>
<td>Praxis™ Extended RAS Panel</td>
<td>Illumina, Inc, San Diego, California, USA</td>
</tr>
<tr>
<td>Stanford Solid Tumor Actionable Mutation Panel (STAMP)</td>
<td>Molecular Genetic Pathology Laboratory, Stanford University, Stanford, California, USA</td>
</tr>
<tr>
<td>Tempus</td>
<td>Tempus Labs, Inc, Chicago, Illinois, USA</td>
</tr>
<tr>
<td>UCSF500 Cancer Gene Panel</td>
<td>Clinical Cancer Genomics Laboratory, University of California San Francisco, San Francisco, California, USA</td>
</tr>
</tbody>
</table>
Appendix G: List of Other Cancers in “Other” Category in All Studies (not listed on evidence maps)

- Adenoid cystic carcinomas
- Adrenocorticotrophic carcinoma
- Anaplastic thyroid carcinoma
- Basal cell carcinoma
- Biliary tract cancers
- Brain cancer
- Carcinoma of unknown primary
- Cholangiocarcinoma
- Epithelioid hemangioendothelioma
- Esophageal cancer
- Gastric cancer
- Gastroesophageal cancer
- Hepatocellular carcinoma (liver)
- Lymphoma
- Mesothelioma
- Metastatic disease
- Multiple myeloma
- Nasal carcinoma
- Neuroblastoma
- Non-clear cell renal cell carcinoma
- Olfactory neuroblastoma
- Oral, head/neck cancer
- Ovarian cancer
- Pancreatic acinar cell carcinomas
- Pediatric brain cancers
- Pediatric cancers
- Pulmonary large-cell neuroendocrine carcinoma
- Rare tumors
- Salivary gland carcinomas
- Sarcomas
- Testicular germ cell tumors
- Well-differentiated/dedifferentiated liposarcoma
### Figure H-1. Study Count Stratified by Cancer Type and Outcome Reported for Studies Using FoundationOne®

<table>
<thead>
<tr>
<th>Health outcomes</th>
<th>Breast</th>
<th>Lung</th>
<th>Prostate</th>
<th>CRC</th>
<th>Melanoma</th>
<th>Bladder</th>
<th>Kidney and RP</th>
<th>Uterine</th>
<th>Leukemia</th>
<th>Pancreas</th>
<th>Thyroid</th>
<th>Multiple</th>
<th>Other</th>
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<tr>
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<td>16</td>
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<td>0</td>
<td>1</td>
<td>1</td>
<td>18</td>
<td>14</td>
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<td>Actionable mutations</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>27</td>
<td>23</td>
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</tbody>
</table>

Abbreviations: CRC, colorectal cancer; RP, renal pelvis.

This heat map displays the number of studies on each cancer type reporting on each of 3 outcomes (see far left panel) for individuals tested with FoundationOne (original and CDx). Darker shades represent higher numbers of published studies reporting data for each outcome for a given cancer. For “Multiple,” patient inclusion was not limited to a specific cancer type. For “Other,” studies reported on a cancer type not listed in the figure (see Appendix G for full list).

### Figure H-2. Patient Enrollment Stratified by Cancer Type and Outcome Reported for Studies Using FoundationOne®

<table>
<thead>
<tr>
<th>Health outcomes</th>
<th>Breast</th>
<th>Lung</th>
<th>Prostate</th>
<th>CRC</th>
<th>Melanoma</th>
<th>Bladder</th>
<th>Kidney and RP</th>
<th>Uterine</th>
<th>Leukemia</th>
<th>Pancreas</th>
<th>Thyroid</th>
<th>Multiple</th>
<th>Other</th>
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</thead>
<tbody>
<tr>
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<td>15 658</td>
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<td>Management changes</td>
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<td>0</td>
<td>0</td>
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<td>55</td>
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<tr>
<td>Actionable mutations</td>
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<td>3553</td>
<td>18 778</td>
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<td>55</td>
<td>31 106</td>
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</table>

Abbreviations: CRC, colorectal cancer; RP, renal pelvis.

This heat map displays the number of individuals given a diagnosis of each cancer type with reported outcomes in studies for individuals tested with FoundationOne® (original and CDx). Darker shades represent higher numbers of patients included in studies. For “Multiple,” patient inclusion was not limited to a specific cancer type in these studies. For “Other,” studies reported on a cancer type not listed in the figure (see Appendix G for full list).
Figure H-3. Study Count Stratified by Cancer Type and Outcome Reported for Studies Using Guardant360™

<table>
<thead>
<tr>
<th>Health outcomes</th>
<th>Breast</th>
<th>Lung</th>
<th>Prostate</th>
<th>CRC</th>
<th>Melanoma</th>
<th>Bladder</th>
<th>Kidney and RP</th>
<th>Uterine</th>
<th>Leukemia</th>
<th>Pancreas</th>
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<td>9</td>
<td>6</td>
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<tr>
<td>Actionable mutations</td>
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<td>5</td>
<td>0</td>
<td>3</td>
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<td>0</td>
<td>0</td>
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<td>4</td>
<td>0</td>
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<td>10</td>
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</table>

Abbreviations: CRC, colorectal cancer; RP, renal pelvis.
This heat map displays the number of studies on each cancer type reporting on each of 3 outcomes (see far left panel) for individuals tested with Guardant360. Darker shades represent higher numbers of published studies reporting data for each outcome for a given cancer. For “Multiple,” patient inclusion was not limited to a specific cancer type. For “Other,” studies reported on a cancer type not listed in the figure (see Appendix G for full list).

Figure H-4. Patient Enrollment Stratified by Cancer Type and Outcome Reported for Studies Using Guardant360™

<table>
<thead>
<tr>
<th>Health outcomes</th>
<th>Breast</th>
<th>Lung</th>
<th>Prostate</th>
<th>CRC</th>
<th>Melanoma</th>
<th>Bladder</th>
<th>Kidney and RP</th>
<th>Uterine</th>
<th>Leukemia</th>
<th>Pancreas</th>
<th>Thyroid</th>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>4371</td>
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</tr>
<tr>
<td>Actionable mutations</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>243</td>
<td>0</td>
<td>6148</td>
<td>1114</td>
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</table>

Abbreviations: CRC, colorectal cancer; RP, renal pelvis.
This heat map displays the number of individuals diagnosed with each cancer type with reported outcomes in studies for individuals tested with Guardant360. Darker shades represent higher numbers of patients included in studies. For “Multiple,” patient inclusion was not limited to a specific cancer type in these studies. For “Other,” studies reported on a cancer type not listed in the figure (see Appendix G for full list).
# Appendix I: Summaries for Included Studies Testing Impact of Genomically Guided Treatment

## Table I-1. Results for Comparison Studies Assessing Impact of Genomically Guided Treatment

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Comparison</th>
<th>Genes/mutations</th>
<th>Therapies</th>
<th>Outcomes</th>
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<tbody>
<tr>
<td><strong>Biliary cancer</strong></td>
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</tbody>
</table>
| Javle 2016\(^{148}\) Retro cohort | Biliary cancer n = 321
Range 56-62 yrs
Cohort study of different classes of biliary cancer | FGFR-targeted therapy vs non–FGFR targeted therapy | Most frequently altered genes: TP53 (59%), CDKN2A/B (19%), ARID1A (13%), and ERBB2 (16%) | Most pts (92%) received chemotherapy
FGFR-specific targeted therapy (n = 20), BGJ398 (n = 16), pazopanib (n = 1), dovitinib (n = 1), TAS-120 (n = 2) | OS: Pts receiving FGFR-targeted therapy superior to those that received non–FGFR targeted therapy (\(p = .006\))
Borderline significance between the administration of targeted therapies and OS in IHCCA (\(p = .07\)) |
| **Colorectal cancer**      |          |                                                                             |                                                                                  |                                                                             |                                                                                               |
| Kato 2019\(^{80}\) Retrospective observational study | N = 94 pts with CRC
Average age 50 yrs | Matched (n = 17) vs unmatched (n = 18) therapy | TP53 (51% of pts) followed by KRAS (34%), APC (27%), BRAF (16%), PIK3CA (16%), and EGFR (15%) | NR                                                                          | OS: Not reached at 11.1 mos for matched group vs 9.4 mos unmatched (ns)
PFS: 6.1 mos compared with 2.3 mos for pts in the unmatched therapy group (\(p = .079\) for multivariable analysis)
SD: 65% matched pts attained for 6 mos or more
PR or CR vs 31% of pts in the unmatched therapy group (\(p = .060\) in univariable analysis; \(p = .045\) in multivariable analysis addition, pts in the matched therapy group had a median) |
| **Head and neck cancers**  |          |                                                                             |                                                                                  |                                                                             |                                                                                               |
| Porter 2020\(^{136}\)     | 60 pts with head/neck cancer | Targeted/matched (n = 8) vs unmatched (n = 21) therapy | Most common: TP53 (68% pts), PIK3CA (34%), NOTCH1 (20%), ARID1A (15%) | Specific therapies NR                                                                 | Targeted therapy (n = 8; 13%): n = 3 stable disease (37.5%), n = 3 progressive disease (37.5%), and n = 2 not evaluated (25%)
Unmatched therapy (n = 21; 35%):
   - n = 11 stable disease (52.4%), n = 9 progressive disease (42.9%), n = 1 complete response (4.8%) |
<table>
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<tr>
<th>Study</th>
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<tr>
<td><strong>Non–small cell lung cancer</strong></td>
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<td>Singal 2019&lt;sup&gt;64&lt;/sup&gt;</td>
<td>4064 pts with NSCLC Median age 66 yrs</td>
<td>Targeted (n = 575) vs nontargeted (n = 560) therapy</td>
<td>871 pts (21.4%) had alterations in EGFR, ALK, or ROS1</td>
<td>Anti-PD-1, EGFR inhib, ALK inhib, chemotherapy non-platinum regimen, platinum regimen</td>
<td>OS: Significantly better for pts receiving NCCN-targeted therapy vs not; also better for pts receiving EGFR inhibitor vs not (both p &lt; .001)</td>
</tr>
<tr>
<td>Schwaederle&lt;sup&gt;107&lt;/sup&gt; PREDICT</td>
<td>88 pts with lung adenocarcinoma Mean age 66.2 yrs at diagnosis</td>
<td>Matched (n = 25) vs unmatched (n = 27) therapy</td>
<td>Most frequent: ALK (6.8% of pts), EGFR (27.3%), MET (14.8%), KRAS (13.6%), TP53 (44.3%)</td>
<td>All 25 matched pts received FDA-approved drugs: n = 15 (60%) on-label, n = 5 (20%) off-label; except pts matched to EGFR T790M (n = 5) who received osimertinib</td>
<td>PFS: Median longest 14.7 mos for matched pts vs 7.8 mos for never-matched pts (p = .28) OS: Median not reached for matched group (median f/u time of 18.6 mos vs 36.7 mos for never matched; p = .928)</td>
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<td><strong>Pancreatic cancer</strong></td>
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<td>Pishvaian 2018&lt;sup&gt;293&lt;/sup&gt;</td>
<td>640 pts with pancreatic cancer</td>
<td>Matched (n = 17) vs unmatched (n = 18) therapy</td>
<td>Most common: BRCA1/2 or ATM (8.4%), cell-cycle genes CCND1/2/3 or CDK4/6 alterations (8.1%); CDKN2A also cited</td>
<td>From NGS testing: Inhibitors of MEK/ERK, CDK4/6, mTOR, PARP, CDK, WNT, FGFR, alone or in combination Of the 165 pts with highly actionable NGS alterations, nearly all (162; 98%) had FDA-approved therapies. Off-label or clinical trials: ATM: Gemcitabine + nab paclitaxel BRAF: Irintocetan BRCA2: Olaparib See Figure 5B in Pishvaian et al for complete list.</td>
<td>PFS: Matched therapy (n = 17): significantly longer median PFS than for unmatched therapy group (n = 18; PFS = 4.1 vs 1.9 mos; HR, 0.47; 95% CI, 0.24-0.94; p = .03) OS: Borderline significant favoring matched (n = 16) vs unmatched (n = 13) therapy (HR, 0.48; 95% CI, 0.21-1.1; p = .08)</td>
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<td>Study</td>
<td>Patients</td>
<td>Comparison</td>
<td>Genes/mutations</td>
<td>Therapies</td>
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<td>Moore 2019</td>
<td>Salivary gland carcinoma (n = 27) Mean age 59 yrs</td>
<td>Targeted (n = 14) vs conventional treatment (no targeted treatment; n=13) N = 14 had targetable findings from NGS; other pts did not NGS performed before systemic therapy (n = 21) and after failure systemic therapy (n = 6)</td>
<td>MSI high (n = 1), RUNX1 mut (n = 2), SPARC overexp (n = 1), RET fusion (n = 1), ERBB2 ampl (n = 2), ERBB2 ampl (n = 1), ERBB2 ampl (n = 2), ERBB2 ampl (n = 1), PIK3CA mut (n = 3)</td>
<td>Pembrolizumab Azacitidine Nab-paclitaxel Vandetanib Trastuzumab/pertuzumab Trastuzumab/pertuzumab Paclitaxel + trastuzumab/ pertuzumab Carboplatin/paclitaxel + trastuzumab Trastuzumab Alpelisib</td>
<td>Stable disease: 8.5 mos PR: 9.7 mos; ongoing PR: 31.2 mos SD: 14 mos CR: 17 mos; ongoing Prog dis: 2 mos PR: 5 mos; ongoing CR: 6 mos Prog dis: 2.5 mos PR: 5 mos; ongoing Median survival: ns difference targeted vs conventional (p = .18) Toxicity: Grade 1-2 weakness and fatigue with vandetanib, azacytidine, nab-paclitaxel Grade 2 hyperglycemia with alpelisib Grade 3 hematologic toxicity with nab-paclitaxel</td>
</tr>
<tr>
<td>Study</td>
<td>Patients</td>
<td>Comparison</td>
<td>Genes/mutations</td>
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<tr>
<td><strong>Solid tumor cancers</strong></td>
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<td>Sadaps 2018&lt;sup&gt;102&lt;/sup&gt; Retrospective observational study</td>
<td>Solid tumor cancers (n = 600); 313 pts underwent treatment changes</td>
<td>Genomic driven (n = 95) vs nongenomic driven (n = 218)</td>
<td>Most frequently altered genes across all cohorts: TP53, CDKN2A/B, KRAS, ARID1A, IDH1, SMAD4, ERBB2</td>
<td>Most common targets for genomic-driven therapy: HER2 (n = 14), BRAF (n = 12), PIK3CA/PIK3R/PTEN (n = 9), EGFR (n = 8), ALK (n = 5), CDK (n = 5), RET (n = 5)</td>
<td>HER2: Afatinib Pertuzumab + trastuzumab Trastuzumab + pertuzumab Ado-trastuzumab Trastuzumab Trastuzumab + FOLFOX BRAF: Dabrafenib + trametinib Dabrafenib See Table A1 in Sadaps et al for all drugs.</td>
</tr>
<tr>
<td>Grenader 2016&lt;sup&gt;58&lt;/sup&gt;</td>
<td>30 pts with solid tumors</td>
<td>Genomically guided (n = 10) vs unguided (n = 20) therapy</td>
<td>Mutations reported only for pts receiving guided therapy: BRAF (n = 2), ERBB2 (n = 2)</td>
<td>BRAF: Trametinib, vemurafenib/panitumumab ERBB2: Pertuzumab/trastuzumab/paclitaxel, neratinib</td>
<td>PFS: Superior median PFS for genomically guided group (86 days vs 49 days; p = .005; HR = 0.55; 95% CI, 0.37-0.84)</td>
</tr>
<tr>
<td>Radovich 2016&lt;sup&gt;125&lt;/sup&gt;</td>
<td>101 pts with solid tumor cancers</td>
<td>Genomically guided (n = 44) vs unguided (n = 57) therapy</td>
<td>Most frequent mutations: KRAS, FGFR1, HER2</td>
<td>Provided details only on responders: FGFR1: Dovitinib (clinical trial) HER2: Trastuzumab + capecitabine KRAS NR</td>
<td>PFS: Superior median PFS for genomically guided group (86 days vs 49 days; p = .005; HR = 0.55; 95% CI, 0.37-0.84)</td>
</tr>
<tr>
<td>Schwaederle 2016&lt;sup&gt;124&lt;/sup&gt;</td>
<td>347 pts with solid tumor cancers</td>
<td>Matched (n = 87) vs unmatched (n = 93) therapy</td>
<td>TP53 (N = 178), CDKN2A (N = 76), KRAS (N = 63), PTEN (N = 42), EGFR (N = 31)</td>
<td>Examples of matched therapies: Anti-EGFR drugs in the presence of EGFR variants mTOR inhibitors for variants in PTEN/PIK3CA/Akt/mTOR pathway BRAF or MEK inhibitors for RAF or RAS aberrations</td>
<td>PFS: Median survival longer for matched group (4.0 vs 3.0 mos; p = .039; Cox regression) SD: More pts in matched group (≥6 mos PR/CR 34.5% vs 16.1%; p = .020 multivariable or propensity score methods)</td>
</tr>
<tr>
<td>Study</td>
<td>Patients</td>
<td>Comparison</td>
<td>Genes/mutations</td>
<td>Therapies</td>
<td>Outcomes</td>
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| Reitsma 2019<sup>171</sup>  | Solid tumor and hematologic cancers           | Matched therapy or clinical trial (n = 17) vs unmatched therapy and no clinical trial (n = 50) | Alterations directing treatment not previously identified by conventional testing: *ERBB2*, *GNAQ Q209L*, *BRAF V600E*, *KRAS* amplification | *ERBB2*: Trastuzumab + paclitaxel  
*GNAQ Q209L*: Trametinib  
*BRAF V600E*: Oxaliplatin + capecitabine + bevacinumab (clinical trial)  
*KRAS*: Trametinib | OS: Nonsignificant difference between matched and unmatched therapy groups (95% CI, 1.1-24.2 vs 4.6, CI, 0-30.9) |
| Bryce 2017<sup>111</sup>    | Retrieved targeted therapy or continued SoC; n = 141 total pts with solid tumor or hematologic cancers; n = 92 with actionable mutations | Guided (n = 29) vs continuation of SoC (n = 14) Subsets of pts received aCGH and WES | Most common for pts receiving genomic guided therapy: *BRAF, EGFR, ERBB2, PIK3CA* | *BRAF*: Vemurafenib  
*PIK3CA*: Exemestane and everolimus/everolimus alone  
*EGFR*: Cetuximab + afatinib, erlotinib, Cetuximab+5-fluorouracil | 13/29 (45%) of guided therapy group responded. 14/92 pts with actionable mutations (15%) stayed with standard care before exhausting options, with 10/14 (71%) responding to treatment. 35% (34/92) of pts with actionable targets not treated; 65% (22/34) chose comfort measures or passed away. |
| Wheler 2016<sup>149</sup>  | Solid tumor and hematologic cancers Prospective single-arm observational study | N = 188 treated with matched (n = 122) vs unmatched (n = 66) therapy | Most frequent leading to matched therapies: *PIK3CA, PTEN, TP53* | *PIK3CA*: PIK3CA inhibitors, sirolimus everolimus, metformin  
*PTEN*: Everolimus, metformin, temsirolimus, PIK3CA inhibitors  
*TP53*: Bevacizumab, vandetanib, pazopanib, sorafenib, regorafenib | Time-to-treatment failure: Better for matched group (HR = 0.52; 95% CI, 0.36-0.74; p = .0003)  
OS: Better for matched group; borderline significant (p = .08) |

Abbreviations: aCGH, array comparative genomic hybridization; ampl, amplification; CI, confidence interval; CR, complete response; CRC, colorectal cancer; f/u, follow-up; HR, hazard ratio; IHCCA, intrahepatic cholangiocarcinoma; mos, months; mut, mutation; MSI, microsatellite instability; NCCN, National Comprehensive Cancer Network; NR, not reported; ns, nonsignificant; NSCLC, non–small cell lung cancer; overexp, overexpression; PFS, progression-free survival; PR, partial response; prog dis, progressive disease; pts, patients; SD, stable disease; SoC, standard of care; WES, whole-exome sequencing; wks, weeks; yrs, years.
Appendix J: List of Cancers in “Other” Category in Single-Arm Studies

- Adenoid cystic carcinomas
- Anaplastic thyroid carcinoma
- Basal cell carcinoma
- Brain cancer
- Carcinoma unknown primary site
- Epithelioid hemangioendothelioma
- Gastric cancer
- Gastroesophageal adenocarcinoma
- Glioblastoma
- Gliomas
- Liver cancer
- Lymphoma
- Mesothelioma
- Olfactory neuroblastoma
- Sarcomas
Appendix K: List of Tests in “Other” Category in Single-Arm Studies

- Archer™ FusinoPlex™ Solid Tumor Panel (Invitae)
- CANCERPLEX (Kew)
- Columbia Combined Cancer Panel
- GeneTrails® Comprehensive Solid Tumor Panel (Oregon Health & Science University)
- GlioSeq® (University of Pittsburgh Medical Center)
- InVisionFirst®-Lung (Inivata)
- InVisionSeq™ (Inivata)
- JAX ActionSeq™ (The Jackson Laboratory)
- JAX Cancer Treatment Profile™ (The Jackson Laboratory)
- OmniSeq Comprehensive® (Omniseq)
- Stanford Solid Tumor Actionable Mutation Panel (Stanford)
- University of California San Francisco 500 Cancer Gene Panel (University of California San Francisco)
- UW-OncoPlex™ (University of Washington)
### Appendix L: Additional Results From Single-Arm Studies

#### Table L-1. Results for Single-Arm Studies Assessing Impact of Genomically Guided Treatment

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Genes/mutations</th>
<th>Therapies</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boussemart et al 2019&lt;sup&gt;333&lt;/sup&gt;</td>
<td>Melanoma (n = 385)</td>
<td>31 BRAF alterations identified in 29 pts by hybrid capture-based NGS after prior negative result. Clinical outcomes reported for 3 pts.</td>
<td>Dabrafenib + trametinib Sorafenib Cobimetinib</td>
<td>Symptom improvement and shrinkage of metastatic lung nodules after 3 mos of treatment; ongoing Response; ongoing No response; currently on immunotherapy</td>
</tr>
<tr>
<td>Groisberg et al 2018&lt;sup&gt;163&lt;/sup&gt;</td>
<td>Multiple rare tumors (n = 95). Median age is 51 years. 36 pts with at least 1 CAM; 13 pts underwent treatment changes.</td>
<td>Cholangiocarcinoma: BRAF V600E mut (n = 1) Erdheim-Chester disease: BRAF V600E mut (n = 2) Glioblastoma multiforme: BRAF V600E mut (n = 1) Salivary gland adenocarcinoma: BRAF V600E mut (n = 1) AKT1 E17K mut (n = 1) Adenoid cystic carcinoma: PIK3R1 E515fs<em>1 (n = 1) KIT and PDGFRA amp (n = 1) Metastatic breast carcinoma: PIK3R1 Y580fs</em>19 (n = 1) PIK3CA H1047R (n = 1) Ovarian clear cell carcinoma: PIK3CA H1047R (n = 1) Prostate neuroendocrine carcinoma: PTEN loss (n = 1) Lung large-cell neuroendocrine carcinoma: PIK3CA amp (n = 1)</td>
<td>Vemurafenib Vemurafenib Vemurafenib AKT inhibitor AKT inhibitor KIT inhibitor + PDGFRA inhibitor Chemo + temsirolimus Chemo + temsirolimus Chemo + temsirolimus Buparlisib + trametinib</td>
<td>PR PR SD: 3 years PR PR SD x 6 cycles SD x 24 cycles SD x 6 cycles; ongoing SD x 7 cycles SD x 8 cycles PD PD PD</td>
</tr>
<tr>
<td>Cole 2018 et al&lt;sup&gt;165&lt;/sup&gt;</td>
<td>Pediatric brain tumors (n = 88). Median age 10 years. 68 pts with at least 1 CAM; 7 pts underwent treatment changes.</td>
<td>BRAF V600E mut (n = 1)</td>
<td>SMO inhibitor BRAF inhibitor</td>
<td>PR No dis prog &gt;12 mos</td>
</tr>
<tr>
<td>Study</td>
<td>Patients</td>
<td>Genes/mutations</td>
<td>Therapies</td>
<td>Outcomes</td>
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<tr>
<td>Gay 2017 et al158</td>
<td>Olfactory neuroblastoma (n = 28). Mean age 50.9 years. 21 pts with at least 1 CAM; 4 pts underwent treatment changes.</td>
<td><em>PTCH1</em> mut (n = 1) <em>PI3KR2</em> mut (n = 1) <em>CTNNB1/Pten/ARID1A/KD M5C</em> muts (n = 1) <em>KIT</em> amp/AXL-ARHGEF fusion/TP53 loss (n = 1)</td>
<td>Sunitinib Everolimus Pazopanib + docetaxel Sunitinib</td>
<td>SD: 24 mos No dis prog &gt;12 mos SD: 24 mos Response</td>
</tr>
<tr>
<td>Johnson et al 2017170</td>
<td>Solid and hematologic malignancies (n = 103). Median age 53 years. 18 pts underwent treatment changes.</td>
<td>Muts spanned multiple pathways RAS/MAPK, PI3K/mTOR, RTK/GFs, and cell cycle</td>
<td>Include MEKi, CDK4/6i, PI3Ki</td>
<td>SD (n = 5) Response (n = 3) PD (n = 7) (Duration of response or SD: 6 weeks-14 mos)</td>
</tr>
<tr>
<td>Mitri et al 2018174</td>
<td>Solid and hematologic malignancies (n = 38). Median age 65 years. 4 pts underwent treatment changes.</td>
<td>Metastatic HR-positive breast cancer: <em>CDKN2A</em> and <em>PIK3CA</em> muts (n = 1) Metastatic castrate-resistant prostate cancer: <em>CDKN2A</em> and <em>WNT</em> mut (n = 1) Metastatic HR-positive breast cancer: <em>CDKN2A/PIK3CA/PTEN</em> (n = 1) Metastatic HR-positive breast cancer: *PIK3CA/MYC/regional loss chromosome 13</td>
<td>Everolimus Palbociclib + celebrex Abemaciclib + fulvestrant Paclitaxel + trastuzumab + pertuzumab</td>
<td>PR for 10 mos and then change in therapy No response SD for 10 mos and then progression PR; ongoing</td>
</tr>
<tr>
<td>Ichikawa et al 2017365</td>
<td>Gastric cancer (n = 207). Median age 66 years. 141 pts with CAMs. Clinical outcomes reported for 1 pt.</td>
<td><em>ERBB2</em> alt (<em>HER2</em> overexpression confirmed with immunohistochemistry)</td>
<td>Trastuzumab + chemo</td>
<td>CR + achieved curative resection</td>
</tr>
<tr>
<td>Pectasides et al 2018298</td>
<td>Gastroesophageal adenocarcinoma (n = 28; PANGEA cohort). Median age 62 years. 9 pts underwent treatment changes.</td>
<td><em>ERBB2</em> mut <em>EGFR</em> mut</td>
<td>Trastuzumab EGFR inhibitor ABT-806</td>
<td>Near complete resolution of metastatic burden; continuing therapy 13 mos from diagnosis Decrease in tumor burden; continuing therapy 16 mos from diagnosis</td>
</tr>
<tr>
<td>Ugurluer et al 2016127</td>
<td>Mesothelioma (n = 11). Median age 65 years. 4 pts with CAMs.</td>
<td><em>NF2/BAP1/PTC/MYD 88/SETD2</em> mut</td>
<td>Vorinostat (off-label)</td>
<td>OS: 14 mos after therapy initiation</td>
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<tr>
<td>Study</td>
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<tr>
<td>Arshad et al 2019</td>
<td>Gastrointestinal stromal tumor (n = 45; Sylvester Comprehensive Cancer Center institutional cohort). Mean age 63 years.</td>
<td>Most frequent muts: KIT and PDGFRA</td>
<td>Tyrosine kinase inhibitors</td>
<td>ctDNA-driven therapy: 31 pts with OS and 18 pts with PFS 12 mos after ctDNA testing</td>
</tr>
<tr>
<td>Patel et al 2019</td>
<td>Pancreatic ductal adenocarcinoma (n = 112). Median age 68 years. 76 pts with CAMs and 8 pts with treatment changes.</td>
<td>GNAS/KRAS/NF1 muts</td>
<td>Trametinib</td>
<td>Symptom improvement and response &gt; 26 weeks post–treatment initiation</td>
</tr>
<tr>
<td>Goodman et al 2014</td>
<td>Basal cell carcinoma (n = 8). Median age 62 years. 7 pts with high TMB and 4 patients with treatment changes.</td>
<td>High TMB (≥20 muts/megabase; n = 4)</td>
<td>Nivolumab, Nivolumab + vismodegib Pembrolizumab</td>
<td>CR; ongoing (17.6+ mos) PR; 4.2 mos CR; ongoing (8.1+ mos) PD after 2.5 months</td>
</tr>
<tr>
<td>Aggarwal et al 2019</td>
<td>Non–small cell lung cancer (n = 323). Median age 65 years. 42 pts received targeted therapy based on liquid biopsy results.</td>
<td>Most frequent muts: EGFR and MET</td>
<td>Most frequent therapies include osimertinib, crizotinib, and erlotinib</td>
<td>CR (n = 1) PR (n = 19) SD (n = 16) PD (n = 6) Median follow-up is 7 mos</td>
</tr>
<tr>
<td>Sabari et al 2018</td>
<td>Lung cancers (n = 210). Median age 65 years. 46 pts received targeted therapy based on liquid biopsy results.</td>
<td>Most frequent muts: EGFR and MET</td>
<td>Most frequent therapies include osimertinib, crizotinib, and osimertinib + bevacizumab</td>
<td>PR (n = 34) PD (n = 1)</td>
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Abbreviations: amp, amplification; CAM, clinically actionable mutations; CR, complete response; ctDNA, circulating tumor DNA; dis prog, disease progression; mos, months; mut, mutation; HR, hazard ratio; NGS, next-generation sequencing; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; pts, patients; SD, stable disease; TMB, tumor mutational burden.
### Table M-1. Summaries of Modeling Studies with Effectiveness Data

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Study/purpose</th>
<th>Patients/data</th>
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| NSCLC  | Steuten et al 2020<sup>234</sup>  
Purpose: To estimate cost effectiveness of MGPT vs SMGT among pts with advanced NSCLC | 5688 pts with advanced NSCLC (stage IIIB or metastatic) who received MGPT (n = 875) or SMGT (n = 4813)  
Data from EHRs of Flatiron Health database; costs from CMS 2017 fee schedule | Multigene panel vs single-gene testing; tests not specified  
Oral targeted therapies vs immunotherapy, chemotherapy, supportive care only, or enrollment in clinical trial | Multigene panel testing “moderately cost-effective.” 22% pts tested positive for *EGFR* (18.5% MGPT vs 17.3% SMGT) or anaplastic lymphoma kinase (3.6% MGPT vs 3.8% SMGT). An additional 8% MGPT pts were found to have *BRAF*, *RET*, *ROST1*, *HER2*, or *MET* mutations. 21% MGPT pts received targeted therapies vs 19% with SMGT. Expected survival was 1.14 LYS in SMGT vs 1.20 LYS in MGPT. Lifetime total costs were $8814 higher per pt for MGPT. Incremental CE ratio of MGPS vs SMGT was $148 478 per LY gained. |
| NSCLC  | Pennell et al 2019<sup>235</sup>  
Purpose: To assess economic impact of NGS vs single-gene testing among pts with metastatic NSCLC from perspective of CMS and US commercial payers | 2222 total pts: hypothetical cohorts of 2066 Medicare-insured pts and 156 commercially insured pts with mNSCLC  
Unit costs for testing based on 2017 CMS Clinical Lab Fee Schedule using 2 US claims databases | Upfront NGS (all alterations tested at once + *KRAS*) vs sequential SMGT vs exclusionary testing (*KRAS* + sequential testing) vs hotspot panels (*EGFR*, *ALK*, *ROST1*, and *BRAF* tested simultaneously + single-gene tests or NGS for *MET*, *HER2*, *RET*, and *NTRK1*) | NGS testing led to cost savings for both CMS ($1 393 678, $1 530 869, and $2 140 795 less than exclusionary, sequential testing, and hotspot panels, respectively) and commercial payers ($3809, $127 402, and $250 842 less than exclusionary, sequential testing, and hotspot panels, respectively). Increasing NGS-tested pts translated into substantial cost savings for both CMS and commercial payers. Upfront NGS testing identifies many pts with targetable alterations in shortest time. |
| NSCLC  | Signorovitch et al 2019<sup>233</sup>*  
Purpose: To develop a decision analytic model to assess budget impact of increased CGP in advanced NSCLC from a US private payer perspective | 532 pts with advanced NSCLC; 266 underwent genomic testing | Budget impact of increased use of FoundationOne<sup>®</sup> vs non-CGP (mix of conventional molecular diagnostic testing and smaller NGS hotspot panels)  
Model inputs based on published literature (epidemiology and treatment outcomes), real-world data (testing and rates, medical service costs), list prices for CGP and anticancer drugs, and assumptions for clinical trial participation | An increase in CGP among those tested, from 2% to 10%, was associated with $0.02 per member per month budget impact; most ($0.013) was attributable to costs of prolonged drug treatment and survival and $0.005 (25%) to testing cost; testing has small budget impact. Approximately 12 pts would need to be tested with CGP to add 1 LY. |
<table>
<thead>
<tr>
<th>Cancer</th>
<th>Study/purpose</th>
<th>Patients/data</th>
<th>Comparisons/tests</th>
<th>Outcomes/conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSCLC</td>
<td>Yu et al 2018&lt;sup&gt;236&lt;/sup&gt; &lt;br&gt;Purpose: To evaluate budget impact of NGS instead of single-gene testing for tissue-based assessment of aNSCLC from the US health care payer perspective</td>
<td>Hypothetical cohort of 316 pts with advanced NSCLC; 179 (57%) undergo genetic testing &lt;br&gt;Based on annual cohort of pts with newly diagnosed cancer from hypothetical 1-million-member health care plan</td>
<td>NGS vs single-gene testing for tissue-based assessment of aNSCLC from US health care payer perspective &lt;br&gt;Epidemiology and testing costs for EGFR, ALK, ROS1, BRAF, MET, HER2, and RET were from literature</td>
<td>Of 57 pts with activating mutations, single-gene testing identified 35; NGS identified 54. NGS decreased expected testing procedure-related costs to individual health plan payer by $24,651. First-line and maintenance treatment costs increased by $842,205, offset by a $385,000 decrease for second-line treatment and palliative care. 5-year budget impact was minimal: $432,554 ($0.0072/member/month). NGS projected to identify more pts for selection of targeted therapy and clinical trial enrollment and is minimally cost additive.</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Dalal et al 2018&lt;sup&gt;242&lt;/sup&gt; &lt;br&gt;Purpose: To assess the time to BRAF testing, compare the characteristics of tested vs not-tested pts, and describe the costs for sequential vs NGS BRAF testing</td>
<td>1260 pts with newly diagnosed lung cancer tested for BRAF (out of 28,011 identified pts) &lt;br&gt;Data obtained from 2 US administrative claims databases with medical and pharmacy claims based on commercial and Medicare supplemental plans</td>
<td>Time to BRAF testing; costs of individual serial single-gene mutation testing vs NGS testing &lt;br&gt;Tests not identified</td>
<td>BRAF was tested using NGS in 6.6% of cases. Using the average costs of individual mutation tests, total cost of sequential testing for KRAS, EGFR, ALK, ROS1, and BRAF tests was $3763 ($464, $696, $1070, $1127, and $406, respectively) while cost of NGS testing was $2860. NGS associated with cost savings compared to sequential testing of individual mutations.</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Li et al 2015&lt;sup&gt;239&lt;/sup&gt; &lt;br&gt;Purpose: To determine whether an NGS panel of 34 genes would be cost effective compared with single-site BRAF V600 testing for pts with metastatic melanoma</td>
<td>Hypothetical cohorts of pts with metastatic melanoma &lt;br&gt;Test costs taken from the Medicare clinical laboratory fee schedule and Quest Diagnostics; drug costs calculated using the average weighted price in the Redbook and dosages from prescribing information</td>
<td>34-gene Oncovantage NGS panel test vs cobas BRAF V600 single-site mutation test</td>
<td>NGS panel cost US$120,022: 0.721 QALYs per pt vs US$128,965 and 0.704 QALYs for single-site mutation test. The NGS panel strategy cost US$8943 less per pt and increased QALYs by 0.0174 per pt. Sensitivity analyses comparing single-site mutation test strategy with NGS panel showed panel strategy had 90.9% chance of having reduced costs and increased QALYs, with cost of the panel test having minimal effect on incremental cost.</td>
</tr>
</tbody>
</table>

* Test-developer sponsored study.

Study/purpose column: Gray panels denote analysis based on hypothetical cohort.

**Abbreviations:** CE, cost-effectiveness; CGP, comprehensive genomic profiling; CMS, Centers for Medicare & Medicaid Services; LY, life-year; MGPT, multigene panel testing; mNSCLC, metastatic non-small cell lung cancer; NGS, next-generation sequencing; NSCLC, non–small cell lung cancer; pts, patients; SMGT, single-marker genomic testing; QALY, quality-adjusted life-year.

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Appendix N: Ongoing Clinical Trials

The table below lists clinical trials selected from a larger list retrieved by our searches. These trials represent those most relevant to this report (i.e., evaluating the clinical utility of commercial NGS-based genomic tests). The list was current at the time of this report’s literature search (see Methods section for details).

Table N-1. Ongoing Clinical Trials

<table>
<thead>
<tr>
<th>Title</th>
<th>Test name</th>
<th>Study summary</th>
<th>Study type/phase</th>
<th>Completion date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
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<tr>
<td>Molecular profiling in young (&lt;50 years of age) patients with metastatic breast cancer&lt;sup&gt;372&lt;/sup&gt;</td>
<td>FoundationOne® CDx</td>
<td>To characterize genetics of young (&lt;50 years of age) Korean patients with metastatic breast cancer n = 200</td>
<td>Interventional, single group</td>
<td>July 2021</td>
</tr>
<tr>
<td>Guardant360&lt;sup&gt;®&lt;/sup&gt; related clinical outcomes in patients who share medical records—breast cancer (GRECO-B)&lt;sup&gt;397&lt;/sup&gt;</td>
<td>Guardant360&lt;sup&gt;®&lt;/sup&gt;</td>
<td>To observe routine clinical care of patients who have been diagnosed with advanced breast cancer and have undergone genomic testing n = 300</td>
<td>Prospective observational, single group</td>
<td>August 2022</td>
</tr>
<tr>
<td>Real world study using comprehensive genomic data on the next treatment decision making in metastatic breast cancer (HOPE)&lt;sup&gt;398&lt;/sup&gt;</td>
<td>FoundationOne® CDx and Guardant360&lt;sup&gt;®&lt;/sup&gt;</td>
<td>To assess real-world clinical integration of molecular profiling in the management of metastatic breast cancer patients connected through a digital tool n = 600</td>
<td>Prospective observational</td>
<td>July 2024 Not yet recruiting</td>
</tr>
<tr>
<td>Title</td>
<td>Test name</td>
<td>Study summary</td>
<td>Study type/phase</td>
<td>Completion date</td>
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<tr>
<td><strong>Colorectal cancer</strong></td>
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<tr>
<td>BESPOKE study of ctDNA guided therapy in colorectal cancer$^{371}$&lt;br&gt;NCT04264702</td>
<td>Signatera™</td>
<td>To examine the impact of genomic testing on adjuvant treatment decisions for patients with colorectal cancer n = 1000</td>
<td>Prospective observational with case-control analysis</td>
<td>June 2024</td>
</tr>
<tr>
<td>Genetic testing in screening patients with metastatic or unresectable colon or rectal cancer for a COLOMATE trial$^{399}$&lt;br&gt;NCT03765736</td>
<td>Guardant360®</td>
<td>To use liquid biopsy genomic testing to determine the proportion of patients with metastatic colorectal cancer who have actionable mutations and who receive molecularly guided therapy n = 500</td>
<td>Prospective cohort</td>
<td>November 2025</td>
</tr>
<tr>
<td><strong>Gastrointestinal cancers</strong></td>
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<tr>
<td>PLATON—platform for analyzing targetable tumor mutations (pilot study)$^{400}$&lt;br&gt;NCT04484636</td>
<td>FoundationOne® CDx and FoundationOne® Liquid CDx</td>
<td>To assess genomic profiling in gastrointestinal cancer therapy, documenting the frequencies of targetable mutations including tumor mutational burden (TMB) and microsatellite instability status (MSI) Patients with hepatocellular cancer, cholangiocarcinoma, gallbladder cancer, pancreatic cancer, oesophageal cancer, stomach cancer n = 200</td>
<td>Nonrandomized interventional</td>
<td>July 2021 Not yet recruiting</td>
</tr>
<tr>
<td>Title</td>
<td>Test name</td>
<td>Study summary</td>
<td>Study type/phase</td>
<td>Completion date</td>
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<tr>
<td><strong>Leukemia</strong></td>
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<tr>
<td>Assessing minimal residual disease by next-generation sequencing to minimize exposure in people With CLL or SLL who have been treated with venetoclax&lt;sup&gt;401&lt;/sup&gt;</td>
<td>conoSEQ&lt;sup&gt;®&lt;/sup&gt;</td>
<td>To determine if patients treated with venetoclax (alone or in combination with another drug), and who are MRD-negative, can stop treatment with venetoclax and remain off-treatment for 12 months or more. To determine whether study participants remain MRD-negative after they stop treatment with venetoclax. Patients with chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL)</td>
<td>Nonrandomized interventional Phase 2</td>
<td>May 2022</td>
</tr>
<tr>
<td>The clonoSEQ&lt;sup&gt;®&lt;/sup&gt; watch registry&lt;sup&gt;402&lt;/sup&gt;</td>
<td>clonoSEQ&lt;sup&gt;®&lt;/sup&gt;</td>
<td>To determine the impact of the clonoSEQ&lt;sup&gt;®&lt;/sup&gt; test on patient care. Patients with acute lymphoblastic leukemia, adult B-cell, chronic lymphocytic leukemia, multiple myeloma, non-Hodgkin lymphoma</td>
<td>Prospective observational cohort</td>
<td>April 2024</td>
</tr>
<tr>
<td>Title</td>
<td>Test name</td>
<td>Study summary</td>
<td>Study type/phase</td>
<td>Completion date</td>
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<tr>
<td>Achieving value in cancer diagnostics: blood versus tissue molecular profiling—a prospective Canadian study (VALUE)</td>
<td>Guardant360®</td>
<td>To compare blood-based profiling (using the Guardant360 assay) vs standard of care tissue-based profiling in patients with non–small cell lung cancer, within the Canadian system n = 210</td>
<td>Prospective observational cohort</td>
<td>December 2020</td>
</tr>
<tr>
<td>Tumor mutational burden in lung cancer patients (MUBULUC)</td>
<td>FoundationOne® CDx</td>
<td>To investigate TMB assessment of biopsy and surgical tumor samples in first-line treatment lung cancer in routine practice, and implications for predicting efficacy of immune checkpoint inhibitors n = 200</td>
<td>Prospective observational cohort</td>
<td>May 2021 Not yet recruiting</td>
</tr>
<tr>
<td>Plasma molecular profiling in ALK inhibitor resistant NSCLC</td>
<td>Guardant360®</td>
<td>To determine if blood-based comprehensive genomic profiling can provide improved clinical outcomes for Asian patients with ALK inhibitor–resistant non–small cell lung cancer n = 50</td>
<td>Prospective observational cohort</td>
<td>August 2022</td>
</tr>
<tr>
<td>Molecular profiling project</td>
<td>Oncomine™ Focus</td>
<td>To use comprehensive molecular profiling of &quot;actionable&quot; alterations in lung cancer specimens to determine the prevalence of each genetic subtype in the local population n = 50</td>
<td>Prospective observational cohort</td>
<td>December 2022</td>
</tr>
<tr>
<td>Title</td>
<td>Test name</td>
<td>Study summary</td>
<td>Study type/phase</td>
<td>Completion date</td>
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<tr>
<td>Lung cancer</td>
<td>Lung cancer next generation sequencing using the oncomine comprehensive assay (LU-NGS-2) 369 <a href="https://clinicaltrials.gov/ct2/show/NCT03558165">NCT03558165</a></td>
<td>To evaluate the utility and added value of using an NGS panel, the Oncomine Comprehensive Assay v3, to profile stage IV lung cancer patients n = 100</td>
<td>Prospective observational cohort</td>
<td>February 2023</td>
</tr>
<tr>
<td></td>
<td>An observational study to evaluate the clinical utility of the oncomine precision assay within the Exactis Network 368 <a href="https://clinicaltrials.gov/ct2/show/NCT04564079">NCT04564079</a></td>
<td>To evaluate the clinical utility of the Oncomine Precision Assay for treating patients with advanced or metastatic non–small cell lung cancer within the Exactis Network n = 200</td>
<td>Prospective observational cohort</td>
<td>April 2023</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>DNA sequencing-based monitoring of minimal residual disease to predict clinical relapse in aggressive B-cell non-Hodgkin lymphomas 406 <a href="https://clinicaltrials.gov/ct2/show/NCT02633111">NCT02633111</a></td>
<td>To assess effectiveness of the Adaptive clonoSEQ® MRD assay for detecting clinical relapse in aggressive B-cell non-Hodgkin lymphoma compared with conventional approaches n = 501</td>
<td>Prospective observational cohort</td>
<td>October 2021</td>
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<tr>
<td></td>
<td>Molecular monitoring with circulating tumor DNA and nivolumab maintenance 407 <a href="https://clinicaltrials.gov/ct2/show/NCT03311958">NCT03311958</a></td>
<td>To determine if maintenance with nivolumab, for patients with diffuse large B-cell lymphoma and high risk of relapse, can convert positive ctDNA to negative ctDNA and/or result in relapse free survival (RFS-ctDNA) of 9 months or longer after positive ctDNA was documented n = 15</td>
<td>Interventional, single-group assignment Early phase 1</td>
<td>April 2022</td>
</tr>
<tr>
<td>Title</td>
<td>Test name</td>
<td>Study summary</td>
<td>Study type/phase</td>
<td>Completion date</td>
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</tr>
<tr>
<td><strong>Multiple cancer types</strong></td>
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<tr>
<td>TCF-001 TRACK (target rare cancer knowledge) study[^408] NCT04504604</td>
<td>FoundationOne® CDx and FoundationOne® Liquid CDx</td>
<td>To establish whether patients with rare tumors (cholangiocarcinoma, cancer of unknown primary site) can benefit from matched molecular therapy as dictated by their next-generation sequencing results; n = 400</td>
<td>Nonrandomized interventional, factorial assignment</td>
<td>December 2022; Not yet recruiting</td>
</tr>
<tr>
<td>Molecular testing for the MD Anderson Cancer Center personalized cancer therapy program[^373] NCT01772771</td>
<td>Guardant360®</td>
<td>To identify gene mutations that provide personalized cancer therapy options or identify clinical trials most relevant for patients with cancer at MD Anderson. Cancer types include glioma, hematopoietic and lymphoid cell neoplasm, malignant solid neoplasm, melanoma, and sarcoma; n = 12,000</td>
<td>Prospective observational case control</td>
<td>March 2033</td>
</tr>
<tr>
<td>Title</td>
<td>Test name</td>
<td>Study summary</td>
<td>Study type/phase</td>
<td>Completion date</td>
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<tr>
<td><strong>Myeloma</strong></td>
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<tr>
<td>Study to assess for measurable residual disease (MRD) in multiple myeloma patients[^409]</td>
<td>clonoSEQ®</td>
<td>To determine if patients with multiple myeloma who are MRD-negative by multiple modalities (&quot;multimodality MRD-negative&quot;) can safely and effectively discontinue post-transplant maintenance therapy (single-agent lenalidomide, pomalidomide, bortezomib, or ixazomib) after receiving at least 1 year of maintenance therapy</td>
<td>Interventional, single-group assignment</td>
<td>December 2024</td>
</tr>
<tr>
<td><strong>Prostate cancer</strong></td>
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<tr>
<td>Prevalence of HRR-related genes mutations and prognosis in metastatic castration resistant prostate cancer (mCRPC) patients in real world setting (ZENSHIN)[^410]</td>
<td>FoundationOne® CDx</td>
<td>To investigate the prevalence of tissue homologous recombination repair (HRR)-related gene mutations and relate findings to health outcomes in patients with metastatic castration resistant prostate cancer</td>
<td>Prospective observational cohort</td>
<td>December 2020</td>
</tr>
<tr>
<td>Clonal emergence and regression during radium-223 therapy for metastatic prostate cancer[^411]</td>
<td>Guardant360®</td>
<td>To use serial ctDNA analysis of tumor-associated mutations with the Guardant360 test to document the presence of clonal emergence and regression during radium-223 therapy for patients with metastatic prostate cancer</td>
<td>Prospective observational case only</td>
<td>December 2020</td>
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</tbody>
</table>

[^409]: NCT04108624
[^410]: NCT04425200
[^411]: NCT03677076
<table>
<thead>
<tr>
<th>Title</th>
<th>Test name</th>
<th>Study summary</th>
<th>Study type/phase</th>
<th>Completion date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid tumor cancers</td>
<td>Testing whether cancers with specific mutations respond better to glutaminase inhibitor, telaglenastat hydrochloride, anti-cancer treatment, BeGIN study[^412]</td>
<td>NCT03872427</td>
<td>Oncomine</td>
<td>Study summary: To determine the efficacy of glutaminase inhibitor telaglenastat hydrochloride (CB-839 HCl) for treating patients with specific genetic mutations in malignant or unresectable solid tumors or malignant peripheral nerve sheath tumors. Patients with advanced malignant solid neoplasm, metastatic malignant solid neoplasm, NF1 mutation positive malignant peripheral nerve sheath tumor, unresectable malignant solid neoplasm. n = 108</td>
</tr>
<tr>
<td></td>
<td>A molecularly guided anti-cancer drug off-label trial (MEGALiT)[^413]</td>
<td>NCT04185831</td>
<td>FoundationOne® CDx</td>
<td>Study summary: To test the safety and efficacy of comprehensive genomic profiling on solid tumor cancers for treatment decisions and to compare 2 different sequencing, bioinformatics, and decision-making platforms; to evaluate the efficacy and safety of off-label treatments based on genomic test results. Patients with solid tumor; to be treated with atezolizumab, everolimus, niraparib, cobimetinib. n = 154</td>
</tr>
</tbody>
</table>

[^412]: http://example.com/nct03872427
[^413]: http://example.com/nct04185831

**Abbreviations:** CDx, companion diagnostic; MRD, minimal residual disease; NGS, next-generation sequencing; CLIA, clinical laboratory improvement amendments; ctDNA, circulating tumor DNA; MRD, minimal residual disease; NSCLC, non-small cell lung cancer; RNA-seq, RNA sequencing
Appendix O: Frameworks for Characterizing Actionable Mutations

OncoKB (Oncology Knowledge Base) is a precision knowledge base containing information on treatment implications of specific biomarkers (see the OncoKB website). The database is organized hierarchically by gene, alteration, indication, and level of evidence, and it annotates the significance of somatic molecular alterations. The potential treatment implications are stratified by the level of evidence that a specific biomarker is predictive of drug response on the basis of FDA labeling, National Comprehensive Cancer Network (NCCN) guidelines, disease-focused expert group recommendations, and scientific literature. Biomarkers are designated by 8 levels (1, 2, 3A, 3B, 4, R1, and R2, and R3) and are broadly categorized as standard care, investigational, or hypothetical targets. A dedicated panel of physicians and cancer biologists review and edit biomarker-associated investigational therapeutic strategies.386 Table O-1 below provides a summary of the different levels of evidence, characteristics of evidence supportive of each level, and clinical implication for treatment.
Table O-1. OncoKB Framework for Characterizing Clinical Actionability of Molecular Targets

<table>
<thead>
<tr>
<th>Level of evidence designation</th>
<th>Type(s) of supporting evidence</th>
<th>Clinical implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>“FDA-recognized biomarker predictive of response to an FDA-approved drug in this indication”</td>
<td>Standard therapeutic implications</td>
</tr>
<tr>
<td>2</td>
<td>“Standard care biomarker predictive of response to an FDA-approved drug in this indication”</td>
<td>Standard therapeutic implications</td>
</tr>
<tr>
<td>3A</td>
<td>“Compelling clinical evidence supports the biomarker as being predictive of response to a drug in this indication, but neither biomarker nor drug is standard care”</td>
<td>Investigational therapeutic implications</td>
</tr>
<tr>
<td>3B</td>
<td>“Compelling clinical evidence supports the biomarker as being predictive of response to a drug in another indication, but neither biomarker nor drug is standard care”</td>
<td>Investigational therapeutic implications</td>
</tr>
<tr>
<td>4</td>
<td>“Compelling biologic evidence supports the biomarker as being predictive of response to a drug, but neither biomarker nor drug is standard care”</td>
<td>Hypothetical therapeutic implications</td>
</tr>
<tr>
<td>R1</td>
<td>“Standard care biomarker predictive of resistance to an FDA-approved drug in this indication”</td>
<td>Standard therapeutic implications</td>
</tr>
<tr>
<td>R2</td>
<td>“Compelling clinical evidence supports the biomarker as being predictive of resistance to a drug, but neither biomarker nor drug is standard care”</td>
<td>Hypothetical therapeutic implications</td>
</tr>
<tr>
<td>R3</td>
<td>“Compelling biologic evidence supports the biomarker as being predictive of resistance to a drug, but neither biomarker nor drug is standard care”</td>
<td>Hypothetical therapeutic implications</td>
</tr>
</tbody>
</table>

Abbreviations: NCCN, National Comprehensive Cancer Network.

*Includes biomarkers recommended as standard care by NCCN or other expert panels but not necessarily recognized by FDA for a particular indication.

The European Society for Medical Oncology (ESMO) unified framework, the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT), aims to aid oncologists in prioritizing potential targets for clinical use by classifying targets for precision medicine according to evidence of clinical utility. The ESMO scale of actionability has 6 tiers (I, II, III, IV, V, X) detailing biomarker-drug combinations based on strength-of-evidence evaluations from clinical studies (see Table O-2 below). The framework also aims to help clinicians therapeutically prioritize genomic alterations described in tumor profiling reports to decrease the chance for misinterpretation of results leading to missed opportunities for effective treatment or over-interpretation of hypothetical targets.
Table O-2. ESMO Scale for Characterizing Clinical Actionability of Molecular Targets

<table>
<thead>
<tr>
<th>Level of evidence designation</th>
<th>Type(s) of supporting evidence</th>
<th>Clinical implications</th>
</tr>
</thead>
</table>
| I: “Alteration-drug is associated with improved outcome in clinical trials” | I-A: “Prospective, randomised clinical trials show the alteration-drug match in a specific tumour type results in a clinically meaningful improvement of a survival end point”  
I-B: “Prospective, non-randomised clinical trials show that the alteration-drug match in a specific tumour type, results in clinically meaningful benefit as defined by ESMO MCBS 1.1”  
I-C: “Clinical trials across tumour types or basket clinical trials show clinical benefit associated with the alteration-drug match, with similar benefit observed across tumor types” | “Access to the treatment should be considered standard care” |
| II: “Alteration-drug match is associated with anti-tumour activity, but magnitude of benefit is unknown” | II-A: “Retrospective studies show patients with the specific alteration in a specific tumour type experience clinically meaningful benefit with matched drug compared with alteration-negative patients”  
II-B: “Prospective clinical trial(s) show the alteration-drug match in a specific tumour type results in increased responsiveness when treated with a matched drug, however, no data currently available on survival end points” | “Treatment should be considered ‘preferable’ in the context of evidence collection either as prospective registry or as a prospective clinical trial” |
| III: “Alteration-drug match is suspected to improve outcome based on clinical trial data in other tumour type(s) or with similar molecular alteration” | III-A: “Clinical benefit demonstrated in patients with the specific alteration (as tiers I and II above) but in different tumour type. Limited/absence of clinical evidence available for the patient-specific cancer type or broadly across cancer types”  
III-B: “An alteration that has a similar predicted functional impact as an already studied tier I abnormality in the same gene or pathway, but does not have associated supportive clinical data” | “Clinical trials to be discussed with patients” |
| IV: “Pre-clinical evidence of actionability” | IV-A: “Evidence that the alteration or a functionally similar alteration influences drug sensitivity in preclinical in vitro or in vivo models”  
IV-B: “Actionability predicted in silico” | “Treatment should ‘only be considered’ in the context of early clinical trials. Lack of clinical data should be stressed to patients” |
| V | “Prospective studies show that targeted therapy is associated with objective responses, but this does not lead to improved outcome” | “Clinical trials assessing drug combination strategies could be considered” |
| X | “No evidence that the genomic alteration is therapeutically actionable” | “The finding should not be taken into account for clinical decision” |

Abbreviations: ESMO, European Society for Medical Oncology.
Appendix P: Additional Evidence on Guided vs Unguided Therapy

Table P-1. Summary of Studies on Genomic-Guided vs Unguided Therapy, Not Included in Evidence Maps

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients/test</th>
<th>Study design/comparison</th>
<th>Major findings</th>
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<tr>
<td>Kato et al 2020&lt;sup&gt;414&lt;/sup&gt;</td>
<td>N = 429 patients with various advanced metastatic cancers NGS with blood/tissue samples, specific IHC/RNA expression, including for immune biomarkers</td>
<td>Retrospective review of RWD on MTB decisions MTB-guided treatment vs physician choice</td>
<td>86 of 429 patients (20%) matched to all drugs recommended by MTB, including combinatorial approaches, while 163 (38%) received physician’s choice (all clinicians saw MTB results). Patients receiving MTB-recommended treatment had significantly longer OS and PFS than did those receiving physician’s choice.</td>
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<td>Lee et al 2019&lt;sup&gt;415&lt;/sup&gt;</td>
<td>N = 715 patients with gastric cancer NGS with AZ300 or Guardant360&lt;sup&gt;®&lt;/sup&gt;; IHC</td>
<td>Prospective cohort Biomarker-assigned drug treatment vs chemotherapy</td>
<td>Patients with biomarker-assigned treatment had better OS and PFS compared with conventional chemotherapy.</td>
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<td>Seeber et al 2019&lt;sup&gt;416&lt;/sup&gt;</td>
<td>N = 161 patients with heavily pretreated cancer Multisite molecular profiling with Caris Molecular Intelligence: sanger sequencing, NGS (hotspot and large panel), IHC, and ISH</td>
<td>Pooled analysis from 3 pilot studies and 21 additional patients Molecular-based treatment vs previous therapy</td>
<td>PFS significantly improved with molecular-based treatment options (120.0 vs 89.5 days).</td>
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<td>Stockley et al 2016&lt;sup&gt;417&lt;/sup&gt;</td>
<td>N = 1640 patients with advanced solid tumors Targeted NGS RUO panels (n = 813), PTEN IHC (n = 788), or MALDI-TOF MS hotspot (n = 827)</td>
<td>Prospective cohort Genotype-matched vs genotype-unmatched trials</td>
<td>Overall response rate higher in patients treated on genotype-matched trials (19%) compared with genotype-unmatched trials (9%; p &lt; .026). Median f/u: 18 months</td>
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<td>Le Tourneau et al 2015&lt;sup&gt;418&lt;/sup&gt;</td>
<td>N = 195 patients with any tumor type; limited to patients with alterations in 1 of 3 molecular pathways (hormone receptor, PI3K/AKT/mTOR, RAF/MEK) NGS (RUO tests), copy number alterations (Cytoscan HD), IHC</td>
<td>Randomized open-label phase 2 trial Molecularly matched treatment vs physician choice; used therapies only off-label</td>
<td>Median PFS not significantly different between molecularly matched and physician choice groups. Median f/u: 11.3 months</td>
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</table>

Abbreviations: f/u, follow-up; IHC, immunohistochemistry; ISH, in situ hybridization; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MS, microsatellite; MTB, molecular tumor board; NGS, next-generation sequencing; OS, overall survival; PFS, progression-free survival; RNA, ribonucleic acid; RUO, research use only; RWD, real-world data.