The Current State of Molecular Testing in the Treatment of Patients With Solid Tumors, 2019

Wafik S. El-Deiry, MD, PhD, FACP[1]; Richard M. Goldberg, MD[2]; Heinz-Josef Lenz, MD, FAPC[3]; Anthony F. Shields, MD, PhD[4]; Geoffrey T. Gibney, MD[5]; Antoinette R. Tan, MD, MHSc[6]; Jubilee Brown, MD[7]; Burton Eisenberg, MD[8,9]; Elisabeth I. Heath, MD, FACP[10]; Surasak Phuphanich, MD[11]; Edward Kim, MD, FACP, FASCO[12]; Andrew J. Brenner, MD, PhD[13]; John L. Marshall, MD [14]

[1]Associate Dean for Oncologic Sciences, Warren Alpert Medical School; Director, Joint Program in Cancer Biology, Brown University and the Lifespan Cancer Institute; Professor of Pathology & Laboratory Medicine and Professor of Medical Science, Brown University, Providence, RI; [2]Professor of Medicine and Director, West Virginia University Cancer Institute, Morgantown, WV; [3]Professor of Medicine, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA; [4]Professor of Oncology, Karmanos Cancer Institute, Detroit, MI; [5]Associate Professor of Medicine, Co-Leader of the Melanoma Disease Group, Lombardi Comprehensive Cancer Institute, MedStar Georgetown Cancer Institute, Washington, DC; [6]Co-Director of Phase I Program, Department of Solid Tumor Oncology and Investigational Therapeutics, Levine Cancer Institute, Atrium Health, Charlotte, NC; [7]Professor and Associate Director of Gynecologic Oncology, Levine Cancer Institute, Atrium Health, Charlotte, NC; [8]Professor of Clinical Surgery, University of Southern California, Los Angeles, CA; [9]Executive Medical Director, Hoag Family Cancer Institute, Newport Beach, CA; [10]Professor of Oncology and Medicine, Karmanos Cancer Institute, Detroit, MI; [11]Professor of Neurology, Director, Division of Neuro-Oncology, Barrow Neurological Institute, Phoenix, AZ; [12]Chair, Solid Tumor Oncology and Investigational Therapeutics, Levine Cancer Institute, Atrium Health, Charlotte, NC; [13]Associate Professor of Medicine, Mays Cancer Center at University of Texas Health San Antonio Cancer Center, San Antonio, TX; [14]Professor of Medicine and Oncology, Director, Ruesch Center for the Cure of Gastrointestinal Cancers, Lombardi Comprehensive Cancer Institute, MedStar Georgetown Cancer Institute, Washington, DC.

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Abstract: The world of molecular profiling has undergone revolutionary changes over the last few years as knowledge, technology, and even standard clinical practice have evolved. Broad molecular profiling is now nearly essential for all patients with metastatic solid tumors. New agents have been approved based on molecular testing instead of tumor site of origin. Molecular profiling methodologies have likewise changed such that tests that were performed on patients a few years ago are no longer complete and possibly inaccurate today. As with all rapid change, medical providers can quickly fall behind or struggle to find up-to-date sources to ensure he or she provides optimum care. In this review, the authors provide the current state of the art for molecular profiling/precision medicine, practice standards, and a view into the future ahead. **CA Cancer J Clin 2019;69:305-343.**

Keywords: biomarkers, cancer, gene expression profiling, drug target, molecular-targeted therapy, molecular profiling, mutation, precision medicine, sequence analysis

Molecular Testing and Its Evolution

Comprehensive molecular profiling of patient tumors has been widely studied over the last few years in a variety of cancers, leading to the development of a new discipline termed "personalized" or "precision" medicine. Molecular profiling is becoming standard practice for most patients with advanced disease, replacing the historical treatment paradigm of prescribing standard chemotherapy based upon the tumor's organ of origin, histology, and stage. This approach has allowed oncologists to reorganize the way they think about cancer and to make treatment recommendations based upon genomic drivers of tumorigenesis. In some cases, this has produced dramatic, positive outcomes, including complete remissions, even in the setting of treatment-refractory disease, delighting both patients and their caregivers.

The molecular profiling field is evolving rapidly. We are now shifting our focus from a few small, predictive, disease-specific, evidence-based tests—chosen "a la carte"—to broader panel testing that measures levels of *or* changes in myriad "genes or gene products." These genomic changes can serve as biomarkers of both response prediction (indicating tumor and patient outcome/response to a specific therapy) and a patient's prognosis (describing innate tumor aggressiveness, which aligns with patient survival regardless of treatment received). Increasing numbers of biomarkers have been identified for which targeted drugs are being discovered and exploited therapeutically. Scientific advances go hand-in-hand with technological advances, which lead to improved therapeutic choices, all of which have garnered US Food and Drug Administration (FDA), Centers for Medicare &

Medicaid Services (CMS), and insurance company attention. Growing acceptance of evidence-based biomarker testing for the purpose of targeting treatment to solid tumors has ensued. Notably, Foundation Medicine's FoundationOne CDx assay, which tests for several well-known markers using next-generation sequencing (discussed later in this review), was recently approved by the FDA and concurrently accepted by the CMS.[1,2]

To facilitate cancer therapy, it is important to distinguish between germline abnormalities and somatic abnormalities. A very good example of this is the recently incorporated BReast CAncer gene (*BRCA*) germline testing for all patients with pancreatic cancer. Germline testing involves an extensive coverage of *BRCA*, whereas current somatic testing covers only certain regions of that gene. As mutation analysis evolves into whole exome sequencing, coverage of germline and somatic testing will be similar if not identical. Given the increased need for somatic testing in patients with pancreatic cancer, it is possible that whole exome sequencing will replace germline testing in guidelines to come. As these "standard" tests evolve, they make the choices facing patients and providers more complex while providing hope that harnessing this knowledge will translate into substantial benefits for patients, including cancer cures and prevention.

Molecular Profiling and Its Methodology

Molecular profiling refers to the assessment of DNA, RNA, and/or proteins within an individual patient's cancer using cells obtained from a tumor biopsy or through the capture of tumor cells circulating in the bloodstream, with the latter being less well established as a methodology. The term "molecular profiling" was initially applied to DNA analysis but evolved with advances in technology to take on a broader meaning to encompass analyses of RNA and proteins. DNA-level alterations do not necessarily lead to biological alterations, thus making examination at the "multiomic" (transcriptome and proteome) level imperative. This multipronged analysis results in the generation of an inordinate amount of data that can be processed only with the help of bioinformatic methodology. Bioinformaticians combine a host of scientific and mathematical data to create a computer infrastructure that assists in the analysis and interpretation of biological data and picks out correlations between certain gene mutations and response to a specific therapy.[3] Currently used molecular profiling techniques are as follows:

DNA and RNA

- *Polymerase chain reaction* (PCR) is used to amplify and detect DNA and RNA sequences. Standard PCR involves the amplification of one or more copies of a chosen DNA sequence to produce millions of copies and enable detection and analysis. Reverse transcription PCR converts RNA templates into complementary DNA for molecular analysis.
- *In situ hybridization* (ISH) localizes and determines a specific DNA or RNA sequence in a tissue section (in situ) or in circulating tumor cells using a labeled complementary DNA, RNA, or modified nucleic acid strand probe. This technique detects gene deletions, amplifications, translocations, and fusions. Gene fusions commonly occur in epithelial cancers as a result of genomic rearrangements or abnormal mRNA processing. ISH techniques include chromogenic ISH and fluorescence in situ hybridization (FISH).

  Chromogenic in situ hybridization (CISH) uses brightfield microscopes for label detection.

  FISH uses fluorescence microscopes for label detection.
- *Sanger sequencing* examines strands of DNA to identify mutations by analyzing long, contiguous sequencing reads. This DNA sequencing takes place according to the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication. This was the primary sequencing method used for well over 20 years and, although it is still widely used, next-generation sequencing (NGS) is now preferred for multigene/variant assessment.

Corresponding author: John L. Marshall, MD, Ruesch Center for the Cure of GI Cancers, Lombardi Comprehensive Cancer Institute, MedStar Georgetown, 3800 Reservoir Road NW, Washington, DC 20007; marshalj@georgetown.edu

All authors are members of the Precision Oncology Alliance.

DISCLOSURES: Wafik El-Deiry is the Scientific Founder and Shareholder in Oncoceutics, Inc as well as p53-Therapeutics, Inc.; he is also cochair of the Caris Life Sciences Precision Oncology Alliance but receives no financial support from the company for research in connection with this role. Richard M. Goldberg reports travel support to consortium meetings from Caris Life Sciences and consulting on new product development for Taiho Pharmaceuticals, Novartis, and Merck. Heinz-Josef Lenz reports grants, personal fees, and travel expenses from Bayer and personal fees from Merck Kg, Bristol-Myers Squibb, and Genentech/Roche outside the submitted work. Anthony F. Shields reports research funding, personal fees, and travel expenses from Caris Life Sciences during the current study; research funding from Taiho Pharmaceuticals, Bayer, Boehringer Ingelheim, Plexicon, Eisai, H3 Biomedicine, Exelisis, Xencor, Lexicon, Daiichi Sankyo, Torque, Halozyme, Incyte, and LSK BioPharma outside the submitted work; personal fees and travel expenses from GE Health care outside the submitted work; and research funding, personal fees, and travel expenses from TransTarget and Inovio Pharmaceuticals outside the submitted work. Geoffrey T. Gibney reports personal fees from Novartis, Genentech, Merck, Bristol-Myers Squibb, Array Biopharma, Jounce, and Newlink Genetics outside the submitted work. Antoinette R. Tan reports travel expenses from Caris Life Sciences during the current study and grants from Merck, Tesaro, Pfizer, and Genentech outside the submitted work. Jubilee Brown reports personal fees from Clovis, Tesaro, AstraZeneca, Biodesix, Caris Life Sciences, and Olympus outside the submitted work. Elisabeth I. Heath reports personal fees and honoraria from Dendreon during the conduct of the study; personal fees and travel expenses from Bayer, Sanofi, Seattle Genetics, Agensys Inc, and Sanofi outside the submitted work; grants and travel expenses from Caris Life Sciences outside the submitted work. Edward Kim reports grants from Roche, Boehringer Ingelheim, Pfizer, AstraZeneca, Merck, and Takeda outside the submitted work. John L. Marshall served as the interim Chief Medical Officer and is the ongoing Director of the Caris Life Sciences Precision Oncology Alliance; he reports personal fees from the company during the course of the study; and he reports grants and personal fees from Bayer, Celgene, Taiho Pharmaceuticals, and Merck outside the submitted work. All remaining authors report no conflicts of interest.

doi: 10.3322/caac.21560. Available online at cacancerjournal.com

- *NGS* is a high-throughput technique that rapidly examines and more broadly detects DNA mutations (often used for circulating tumor DNA), copy number variations (CNVs), and gene fusions (using an RNA sequencing panel) across the genome. NGS can be performed on a range of cancer types using blood, solid tissue, and bone marrow samples. Precise tissue collection and workup are necessary for accurate results. Laboratory regulatory agencies constantly provide updated guidance documents pertaining to the design, development, and use of NGS-based tests, recognizing the importance of NGS in cancer diagnostics and therapeutics.
- *Pyrosequencing* detects and quantifies mutations, methylation, etc, through sequencing by synthesis—a method that performs DNA sequencing by detecting the nucleotide that is incorporated by DNA polymerase.
- *Fragment analysis* detects changes in DNA (eg, the length of a specific DNA sequence) or RNA to indicate the presence or absence of an inserted or deleted genomic sequence.

Protein

- *Immunohistochemistry (IHC)* uses the principles of antibody binding to proteins to determine the levels of protein expression in tissue samples. Tumor-related proteins of interest can include tumor-specific antigens, protein products of oncogenes and tumor suppressor genes, tumor cell proliferation markers, and enzymes.

Molecular Profiling Assays and Why Physician Oncologists and Pathologists Should Be Familiar With Them

Modern approaches to tumor profiling assess DNA, RNA, and proteins to form a detailed molecular map to guide more precise and individualized treatment decisions. Because the field of molecular profiling is continually evolving, physician education is vital. Clinical oncologists and pathologists benefit greatly from an understanding of the technology involved, possibly even gaining hands-on experience in molecular profiling assays and their interpretation. Any treating physician should know what, when, and how to test and how to make subsequent informed, patient-personalized treatment decisions.[4,5] Correct interpretation of profiling results is critical; many fear that overinterpretation or misinterpretation will lead to treatment of patients with ineffective but expensive therapies, negatively affecting not only patient lives but also the health care budget. Laboratories offering broad molecular profiling services should be suitably Clinical Laboratory Improvement Amendments (CLIA)–certified for this exact purpose to ensure quality control (see CLIA-approved laboratories offering molecular panel analysis, below). However, even CLIA-certified laboratories do not use identical methodologies and techniques, which can still lead to variable results. Reproducibility is key, and the rationale behind assay cutoff limits should be strong. Even before a patient sample is submitted for profiling, the pathologist or the treating physician—whoever plays the lead role in any particular institution—must ensure quality-controlled tissue sample collection.[6,7] Reputable molecular testing laboratories will advise on the exact set of tumor profiling tests to perform, how to process samples, and how to interpret the final generated report, which is created to inform physicians of treatment choices for their patients. Still, physician education is key to such a critical set of processes.

Biomarker Testing in the Clinic

Targeted therapies are showing efficacy in the right subgroups of patients. Of course, these subgroups must be defined, and this process is becoming more accurate and efficient with evolving molecular testing methods and broader use in research and in the clinic. As this process improves, treatment options will improve for an increasing number of patients while eventually emerging as a more cost-effective, generally beneficial option compared with the currently accepted trial-and-error treatment model.

The biomarker information within Tables 1 through 2.12 is based mainly on the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (NCCN Guidelines), NCCN Biomarkers Compendium (NCCN.org, Accessed February 6, 2019), and FDA recommendations and approvals (for the full definitions of all genes, please see the Supporting Information). Although the NCCN Biomarkers Compendium details not only predictive but also prognostic, diagnostic, screening, monitoring, and surveillance markers, the focus of this current review is on predictive biomarkers that can be used to guide treatment decisions. Within Tables 1 through 2.12, the classifications in the "evidence" columns are based on the level of clinical evidence available and the degree of consensus among NCCN panel and other experts. In some cases, clinical evidence comes from large, well-designed, randomized controlled trials, but in many cases, it is mostly based on data from indirect comparisons among randomized trials, phase 2 or nonrandomized trials, multiple smaller trials, retrospective studies, or merely clinical observations. In some cases, substantial clinical data are lacking and evidence comes from clinical experience alone. On the basis of all these factors and how compelling the data are, the evidence is rated as:

1. Based upon high-level evidence, there is uniform NCCN and other expert consensus that the intervention is appropriate (high-level, wide acceptance).

2A. Based upon lower level evidence, there is uniform NCCN and other expert consensus that the intervention is appropriate (lower level, wide acceptance).

2B. Based upon lower level evidence, there is some NCCN and other expert consensus that the intervention is appropriate (lower level, limited acceptance).

TABLE 1. Predictive Microsatellite Instability/Mismatch Repair Testing for Any Solid Tumor

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
MMR	Expression	See *Microsatellite Instability-High Tumors and DNA Mismatch Repair* in the text	IHC	dMMR and MSI-H tests on available tissue are recommended to predict response to pembrolizumab[a]	Lower level; wide acceptance	All
MLH1, MSH2, MSH6, or *PMS2*	Mutation (= dMMR expression)		NGS	Where applicable, dMMR and MSI-H tests are used together to identify whether a patient should undergo further mutation testing for Lynch syndrome[b]		
MSI	Testing (changes in short repeated DNA sequences)	See *Microsatellite Instability-High Tumors and DNA Mismatch Repair* in the text	PCR, NGS	dMMR and MSI-H tests on available tissue are recommended to predict response to pembrolizumab[a]	Lower level, wide acceptance	All
				Where applicable, dMMR and MSI-H tests are used together to identify whether a patient should undergo further mutation testing for Lynch syndrome[b]		

Abbreviations: dMMR, deficient mismatch repair; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, microsatellite instability-high; NGS, next-generation sequencing; PCR, polymerase chain reaction.
[a]Nivolumab alone or in combination with ipilimumab may also be an option for patients with colorectal cancer.
[b]dMMR is a characteristic feature of Lynch syndrome, which can play a part in patients (particularly younger patients) with cancers of the gastrointestinal tract (particularly colorectal), endometrium, ovary, brain, breast, and renal pelvis. In Lynch syndrome, dMMR leads to insufficient repair of repetitive DNA sequences and thus a higher risk of multiple malignant tumors.

Infrequent but Important Site-Agnostic Biomarkers

Microsatellite instability-high tumors and DNA mismatch repair

Microsatellite instability (MSI) is the result of inactivation of the DNA mismatch repair (MMR) system and is characterized by a high frequency of frameshift mutations in microsatellite DNA. In a portion of tumors, MSI is caused by germline mutations in one of the MMR genes (*MLH1*, *MSH2*, *MSH6*, or *PMS2*), which results in hereditary Lynch syndrome. However, the majority (80%) of MSI cases are sporadic, often because of hypermethylation of the *MLH1* gene promoter.[8,9]

MSI-high (MSI-H) has been found in as many as 24 primary cancer types, most of which are displayed in Table 3,[10,11] and appears to be a generalized cancer phenotype in about 4% of all adult cancers. Tumor MSI-H status is prognostic (patients with early-stage cancers that are MSI-H have a better prognosis than those with microsatellite stable tumors) as well as predictive—many MSI-H tumors are exquisitely sensitive to PD-1/PD-L1 inhibitors.[12,13]

At present, the FDA has granted approval for practitioners to administer the PD-1 inhibitor pembrolizumab for the treatment of patients with unresectable or metastatic, MSI-H or MMR-deficient (dMMR) solid tumors (site-agnostic). Currently, the approval is for patients with tumors that have progressed after prior treatment who have no satisfactory alternative treatment options, as well as for patients with MSI-H or dMMR colorectal cancer (CRC) after progression on a fluoropyrimidine, oxaliplatin, and irinotecan, and in the first line for non–small cell lung cancer (NSCLC).[14,15] In 2017, the FDA granted accelerated approval of single-agent nivolumab, another PD-1 inhibitor, for the treatment of adult and pediatric patients older than 12 years with MSI-H or dMMR CRC. Subsequently, in 2018, the FDA granted accelerated approval to a combination of nivolumab plus ipilimumab (a CTLA-4 inhibitor) for treatment of the same set of patients.[16,17] See Table 1 for MSI/MMR biomarker testing recommendations.

Neurotrophic receptor tyrosine kinase

Members of the neurotrophic receptor tyrosine kinase (NTRK) fusion oncogene family, *NTRK1/NTRK2/NTRK3,* are most prevalent in rare adult cancer types and in several pediatric cancers, although they can occur in a very small proportion (approximately 1%) of commonly occurring cancer types in adults, including NSCLCs, CRCs, head and neck cancers, thyroid cancers, bladder cancers, gliomas, and malignant melanomas (Table 4[18]). *NTRK1*, *NTRK2*, and *NTRK3* fusions and the proteins they encode (neurotrophin receptor kinase A [TRKA], TRKB, and TRKC, respectively) are observed at an increased frequency in highly aggressive cancers such as glioblastoma

TABLE 2.1. Currently Recommended Molecular Testing for NSCLC

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
ALK	Gene fusion	Metastatic workup	FISH, NGS, RT-PCR[a]	Response to oral ALK TKIs; alectinib has improved efficacy over crizotinib in first line	High-level, wide acceptance	Adenocarcinoma, large cell, NSCLC NOS
	Fusion protein expression		IHC[b]			
		Together with *EGFR* testing in "never smokers" or small/mixed histology specimens		Response to oral ALK TKIs, eg, crizotinib	Lower level, wide acceptance	Squamous cell
EGFR T790M	Mutation	Metastatic workup	NGS, multiple mutation testing	Resistant to EGFR TKIs	High-level, wide acceptance	Adenocarcinoma, large cell, NSCLC NOS
EGFR exon 21 (L858R, L861), exon 20 (S768I), exon 18 (G719X, G719)	Mutation	Metastatic workup	NGS, multiple mutation testing	Sensitive to EGFR TKIs	High-level, wide acceptance	Adenocarcinoma, large cell, NSCLC NOS
					Lower level, wide acceptance	Squamous cell
EGFR exon 19	Deletion	Metastatic workup	NGS, multiple mutation testing	Sensitive to EGFR TKIs	High-level, wide acceptance	Adenocarcinoma, large cell, NSCLC NOS
					Lower level, wide acceptance	Squamous cell
EGFR exon 20 7p12	Insertion mutation	Metastatic workup	NGS, multiple mutation testing	Likely resistant to EGFR TKIs	High-level, wide acceptance	Adenocarcinoma, large cell, NSCLC NOS
					Lower level, wide acceptance	Squamous cell
ROS1	Fusion rearrangement	Metastatic workup	NGS, FISH, RT-PCR	Responsive to ROS1 TKIs	Lower level, wide acceptance	Adenocarcinoma, large cell, squamous cell, NSCLC NOS
PD-L1	Protein expression ≥50%	Metastatic workup	NGS, multiple mutation testing	Response to pembrolizumab in first-line; FDA approved treatment[15]	Lower level, wide acceptance	Adenocarcinoma, large cell, NSCLC, squamous cell NOS
KRAS	Mutation	Metastatic workup	Gene sequencing	Resistance to EGFR TKIs. Gives poor prognosis compared with *KRAS* wt	Lower level, wide acceptance	All NSCLC
BRAF	Mutation, V600E	Metastatic workup	NGS, pyrosequencing, AS-PCR	Emerging targeted agents[19]; responsive to combined BRAF and MEK inhibition	Lower level, wide acceptance	All NSCLC
HER2	Mutation	Any time	NGS, multiple mutation testing	Emerging targeted agents[20]	Lower level, limited acceptance	All NSCLC
MET	Amplification, mutation	Any time	NGS, FISH	Emerging targeted agents[21]	Lower level, wide acceptance	All NSCLC
RET	Fusion, rearrangement	Any time	NGS, FISH, RT-PCR	Emerging targeted agents[22,23]	Lower level, wide acceptance	All NSCLC

Abbreviations: AS-PCR, allele-specific polymerase chain reaction; FDA, US Food and Drug Administration; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NGS, next-generation sequencing; NOS, not otherwise specified; NSCLC, non–small cell lung cancer; PD-L1, programmed death 1 ligand; RT-PCR, reverse transcription-polymerase chain reaction; TKIs, tyrosine kinase inhibitors; wt, wild type.
[a]FISH is the US Food and Drug Administration-approved method for *ALK* gene rearrangement. NGS and RT-PCR currently are not used widely in clinical practice.
[b]IHC can be used as a good alternative to FISH.[24]

TABLE 2.2. Currently Recommended Predictive Molecular Testing for Colon and Rectal Cancers

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
KRAS/*NRAS*[a]	Mutation	Workup for metastatic disease (suspected or proven)	NGS[b]	Avoid cetuximab or panitumumab treatment in patients who have tumors with *KRAS* and *NRAS* mutations (exons 2, 3, and 4 in both)	NCCN indicate *lower level, wide acceptance*, but many believe classification is *high-level, wide acceptance*	Metastatic synchronous adenocarcinoma (any T, any N, M1), suspected or documented; *or* Metachronous metastases by CT, MRI, and/or biopsy, documented
BRAF[a]	Mutation V600E	Workup for metastatic disease (suspected or proven)	NGS, pyrosequencing, AS-PCR[b]	Cetuximab or panitumumab treatment is not recommended in patients who have tumors with *BRAF* V600E mutations unless given with a BRAF inhibitor such as vemurafenib The use of irinotecan in combination with cetuximab or panitumumab plus vemurafenib is recommended in all patients with previously treated mCRC	NCCN indicates *lower level, wide acceptance*, but many believe classification is *high-level, wide acceptance*	Metastatic synchronous adenocarcinoma (any T, any N, M1), suspected or documented; *or* Metachronous metastases by CT, MRI, and/or biopsy, documented

Abbreviations: AS-PCR, allele-specific polymerase chain reaction; CT, computed tomography; mCRC, metastatic colorectal cancer; MRI, magnetic resonance imaging; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; PCR, polymerase chain reaction.
[a]*KRAS* and *NRAS* are determined alongside *BRAF* mutations.
[b]Testing can be performed on primary and/or metastatic colorectal tissue specimens.

multiforme, and recognition of their potential oncogenic activity led to the use of this fusion family as a predictive biomarker as well as a drug target.[25]

Larotrectinib is an oral and highly selective TRK inhibitor that was granted accelerated approval by the FDA on November 26, 2018, for the treatment of adult and pediatric patients with metastatic or unresectable solid tumors that have an *NTRK* fusion without a known acquired resistance mutation (*NTRK* kinase domain mutations, including solvent front mutations). Patients must have a cancer that has progressed after treatment and/or have no satisfactory alternative treatment for their disease.[26] The approval of larotrectinib is the second tissue-agnostic FDA approval, after pembrolizumab, for the treatment of cancer.

Another TRK inhibitor named entrectinib (RXDX-101) was granted a breakthrough therapy designation by the FDA in 2017, although it has not yet been approved for use as a treatment for adult and pediatric patients who have *NTRK*-positive, locally advanced or metastatic solid tumors that have either progressed after prior therapies or have no acceptable standard therapy options.[27,28]

NTRK fusion testing has evolved massively over the last year or 2, and new discoveries are constantly being made using a range of different assays. The *NTRK* fusions displayed in Table 4[18] are taken from a study that was originally published in 2018. Although comprehensive at the time, this table does not contain the complete list of fusions known today, in 2019. IHC has been used as an initial screening tool to inform highly sensitive but less available and more expensive molecular testing methodologies.[29-31] However, it is now clear that IHC does not have sufficient sensitivity to detect all existing *NTRK* fusion-encoded proteins, meaning that tumor samples should certainly be assayed using FISH or NGS from the get-go.[18] In conclusion, clinicians need to be aware of all 3 TRK targets and arrange adequate testing for all of them.

Germline alterations and their testing

Gene mutations can be somatic or germline; the former spontaneously occur after birth, and the latter are inherited (ie, present at birth). Tumor genetic (somatic) testing detects mutations that may actually be germline alterations, but germline alterations require confirmation in matched normal samples (eg, DNA extracted from white blood cells, buccal swabs, or cultured skin fibroblasts) from the tumor-bearing host. Suspected germline mutations and genetic testing are relevant to cancer treatment and prevention. There is potential for patients to develop tumors at other

sites or for family members to develop cancer, particularly early-onset malignancies.

Table 5[32] lists the somatic mutations that may be germline. This table indicates the cancer types for which germline testing should be carried out if the specified somatic mutations are found in a patient's tumor profile.

There are 3 main categories of tumor genetic modifications with wide variation in the expectation that these reflect germline changes. The first comprises common tumor mutations associated with rare germline alterations. For example, mutations in *TP53* are found in greater than 60% of lung cancers.[33] Although *TP53* mutations can be inherited in the Li-Fraumeni syndrome, such familial syndromes are rare. It is believed for the most part that there is little need for germline testing unless the personal or family history is suggestive of such a syndrome. The second category comprises moderately common somatic mutations that may be associated with familial syndromes. For example, in colon cancer, dMMR is found by routine MSI or IHC testing in about 12% of tumors.[34] Molecular germline testing demonstrates that about one-quarter of these dMMR alterations are inherited. Hence tumor testing should lead to germline confirmation in patients and possibly further evaluation of family members. The final category comprises uncommon tumor mutations that often reflect germline mutations. As an example, patients with breast and ovarian cancers regularly have germline testing done for *BRCA1* and *BRCA2*, especially if the personal or family history is suggestive. With routine molecular genetic tumor testing, *BRCA1*/*BRCA2* mutations are being found in patients with other tumors where it is less expected. An analysis of 100 patients with pancreatic cancer found that 7 had mutations in *BRCA2*, 4 of which were in the germline.[35] Finding *BRCA1/BRCA2* mutations in the tumor may aid in choosing therapy but requires germline testing for confirmation and consideration of genetic counseling for the family.

It has generally been considered that germline testing is not always needed if somatic tumor testing has been done. However, it must be kept in mind that molecular genetic tumor testing can miss a small percentage of inherited cases, where mutations are outside the hotspots covered in the somatic panel or large-scale deletions and duplications have occurred. Conversely, larger gene panel profiling may actually identify previously unknown, clinically relevant alterations that are germline, either de novo or inherited from parents, despite a lack of associated clinical history.[36]

In conclusion, taking into consideration the increasing availability of germline testing and whole exome sequencing to identify inheritable mutations, as well as the personal and family history of cancer and the potential need for genetic counseling, medical teams can help provide better treatment selection for patients with some types of cancer and help to create a systematic approach to hereditary risk.

Disease-Specific Biomarkers

In the subsections below and in Tables 2.1 through 2.12, we address the currently accepted genes or gene products that act as predictive biomarkers (and risk assessment markers in some cases) for each specific solid tumor. Details on when in the disease course the presence or levels of these markers should be assessed are also included. Under each of the following subsections, we also include some description of pertinent biomarkers in research. Compelling evidence suggests that these biomarkers will be listed in the NCCN "recommended" biomarker category in the foreseeable future.

Lung cancers

Lung cancer therapy continues to follow the genomic testing paradigm (see Table 2.1[15,19-24]). All patients with NSCLC should be tested for *EGFR*, *ALK*, *ROS1*, *BRAF*, and PD-L1 at baseline before treatment. Patients with uncommon mutations of *EGFR* may also be treated with tyrosine kinase inhibitor therapy. Other recommended markers of interest include *EGFR* insertion 20 mutations, *RET* rearrangements, and *MET* exon 14 mutations. All of these targets are still being actively investigated in clinical studies and hold potential for patient treatment.

Gastrointestinal cancers

Colon and rectal cancers. Oncologists now recommend the assessment of several predictive markers in patients with CRCs (see Table 2.2). The ideal time to perform genomic testing for treatment purposes is a matter of some controversy and varies depending on disease stage. At the time of initial diagnosis of a stage I, II, or III tumor, it is reasonable to perform MSI testing. Patients with MSI-H, locally confined tumors have a better prognosis, and recommendations are for patients with MSI-H stage II tumors to forgo adjuvant therapy.[37,38] Additional evidence suggests that 5-fluorouracil (5-FU) and related agents, such as capecitabine, can actually worsen outcomes when delivered as single agents to patients with early-stage MSI-H CRCs.[39,40] Treatment with an oxaliplatin regimen is the standard of care recommended for MSI-H stage III CRCs. Finally, guidelines now recommend universal MSI testing in all stages of CRC to determine whether patients have a germline mutation indicative of Lynch syndrome.[41] If both the tumor DNA and the patient's germline DNA harbor an MMR defect, this indicates that the patient has Lynch syndrome. Oncologists need to refer these patients for genetic counseling and a discussion about potential testing of relatives. Such individuals should have screening for

TABLE 2.3. Currently Recommended Predictive Molecular Testing for Gastric, Esophageal, and Gastroesophageal Junction Cancers

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
HER2	Gene amplification	Workup any time	(F)ISH	Particularly if trastuzumab therapy is being considered	Lower level, wide acceptance	Gastric, esophageal, and gastroesophageal junction cancers
PD-L1 (CD274) and HER2 protein	Expression	Workup any time for suspected or documented, inoperable, locally advanced, recurrent, or metastatic adenocarcinoma	IHC, FISH	HER2-negative status corresponds with higher PD-L1 expression rates; together with MMR, HER2 is a potential biomarker for anti–PD-L1 therapy	Lower level, wide acceptance	Gastric, esophageal, and gastroesophageal junction cancers

Abbreviations: dMMR, deficient mismatch repair; (F)ISH, (fluorescence) in situ hybridization; IHC, immunohistochemistry; MMR, mismatch repair; PD-L1, programmed death-1 ligand.
[a]Patients who have gastric cancer with dMMR and HER2-negative status exhibited higher PD-L1 expression rates. These findings indicate that MMR and HER2 status might be potential biomarkers for anti–PD-L1 therapy. Pembrolizumab treatment is approved for patients whose (gastric) tumors express PD-L1[42] (levels ≥1 using the US Food and Drug Administration–approved IHC test).

Lynch syndrome–associated cancers at an earlier age, and more intensive screening is called for than is recommended for individuals without such a cancer susceptibility mutation. There is additional evidence that the use of aspirin can reduce premalignant polyp formation in patients and their relatives with MSI-H tumors.[43] Aspirin has also been associated with improved outcomes in patients with tumors that harbor *PIK3CA* mutations, suggesting a potential value for assessment of mutations in that gene.[44] MSI testing is also an eligibility requirement for the current US intergroup trial of combined 5-FU, leucovorin, and oxaliplatin (FOLFOX) with or without atezolizumab, a PD-1 inhibitor, in patients with MSI-H stage III colon cancer.

In patients with advanced CRC, MSI testing is also indicated at diagnosis. Mutations in or overexpression of additional genes that are predictive of outcomes include *BRAF, HER2, KRAS, NRAS, NTRK, POLE, PIK3CA, PTEN,* and *RSP03*. Often, other than for *RAS* mutations, the optimal time for this testing is when tumors become refractory to standard chemotherapy so that the assessment reflects the current status of the disease. Patients with MSI-H tumors are now eligible for therapy with PD-1–targeting, PD-L1–targeting, and/or CTLA-4–targeting immunotherapies after their disease becomes refractory to standard chemotherapy. Those with *NTRK* fusions are candidates for treatment with larotrectinib.[26] Individuals whose tumors harbor an *RAS* mutation are insensitive to treatment with and should not receive an anti-EGFR–targeted monoclonal antibody such as cetuximab or panitumumab.[45] It is likely that additional genomic analyses that are currently underway or to be evaluated in future studies, involving whole genome or whole exome sequencing in cohorts of patients with known outcomes, will identify other mutations that have either prognostic or predictive utility.

BRAF as a CRC prognostic factor. *BRAF* mutational status is used as a strong predictor for overall survival (OS) at all stages of disease; patients with *BRAF*-mutated CRC have a generally poor prognosis.[46-52] *BRAF* V600E is the best known mutation assessed using NGS.[53] Compared with patients who have CRC with *BRAF* wild-type tumors, patients whose tumors manifest a *BRAF* mutation are generally older and more likely to be female. Such patients commonly have higher grade cancers at diagnosis, with a primary tumor that is more likely to be right-sided and to have a higher number of cancer-involved lymph nodes. These *BRAF*-mutated tumors are also more likely to be MSI-H.[54]

Gastric, esophageal, and gastroesophageal junction cancers. See Table 2.3.[42]

Pancreatic cancers. See Table 2.4.

Genitourinary cancers

Bladder cancers. In The Cancer Genome Atlas extended 2017 study carried out by Robertson et al, findings from the complete cohort of 412 muscle-invasive bladder cancer cases revealed that mutations in the DNA repair genes *ATM* (n = 57; 14%) and *ERCC2* (n = 40; 10%), and deletions in *RAD51B* (n = 10; 2%) were significant.[55]

It was found that all nonsilent somatic *ERCC2* mutations were missense, and many could be mapped within the conserved helicase domain. Dominant negative effects on ERCC2 function were observed.[56] Thus, bladder cancer missense mutations in *ERCC2* were associated with improved response to cisplatin-based chemotherapy. However, *ERCC2* mutations are distributed across the gene, and the functional impact of most individual *ERCC2* mutations is unknown. Recently, Li et al reported developing a microscopy-based

TABLE 2.4. Currently Recommended Predictive Molecular Testing for Pancreatic Cancers

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
BRCA1 and *BRCA2*	Mutation (somatic and germline)	Initial workup if the patient has a strong family history on initial diagnosis[a]	NGS	A known germline mutation could help guide therapy (eg, PARP and other DDR enzyme inhibitors). In October 2018, olaparib was approved for the treatment of patients with germline *BRCA*-mutated, metastatic pancreatic cancer that has not progressed after first-line, platinum-based chemotherapy	Nine percent of pancreatic cancers harbor a germline or somatic *BRCA1* or *BRCA2* mutation, and this has an impact on response to therapy. *BRCA* testing in patients who are still responsive to cytotoxic therapy is becoming standard practice. The use of PARP inhibitors, specifically olaparib, in these patients is an option	Pancreatic adenocarcinoma

Abbreviations: DDR, DNA damage repair; NGS, next-generation sequencing; PARP, poly(adenosine diphosphate-ribose) polymerase.
[a]Having at least one close relative with prostate cancer (possibility of germline mutations) and/or at least one close relative with breast, ovarian, or pancreatic cancer (possibility of a *BRCA2* germline mutation) or with colorectal, endometrial, gastric, ovarian, pancreatic, small bowel, urothelial, kidney, or bile duct cancer (possibility of Lynch syndrome through germline mutations in *MLH1*, *MSH2*, *MSH6*, or *PMS2*) is a risk-factor.

assay that measures the nucleotide excision repair function of clinically observed *ERCC2* mutations. Most helicase domain mutations impaired the function. In addition, a preclinical *ERCC2*-deficient bladder cancer model showed that *ERCC2* loss was sufficient to drive cisplatin sensitivity. Thus, *ERCC2* was concluded to be a predictive biomarker in bladder cancer. Moreover, this study underscores the importance of combining genomic and functional approaches in a co-clinical trial to guide precision oncology for conventional chemotherapy agents. Current evidence presented here supports the idea that *ERCC2* and *ATM* are potentially useful markers in muscle-invasive bladder cancer.[55-57]

Prostate cancers. It was recently reported that patients with metastatic castration-resistant prostate cancer (mCRPC) harboring germline mutations in *BRCA1/BRCA2* and *ATM* have superior clinical outcomes after first-line treatment with abiraterone and enzalutamide (see Table 2.5).[58] The authors suggested that this improved response is likely driven by mutations in *BRCA1*, *BRCA2*, and *ATM*. Because these conclusions were based on only 9 patients harboring *BRCA/ATM* germline mutations and the study was not entirely prospective, these findings require prospective validation in larger patient cohorts. A separate, small, retrospective study found that all responders to poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitor therapy harbored *BRCA2* mutations, whereas nonresponders did not.[59] However, it was agreed that the functional relevance of mutations in DNA repair genes other than *BRCA2* should be considered before committing to PARP inhibitor therapy.

At the American Society of Clinical Oncology 2018 meeting, De Bono et al[60] reported preliminary findings from the KEYNOTE-199 phase 2 trial comparing responses to the immune checkpoint inhibitor pembrolizumab in patients who had mCRPC with or without tumor expression of PD-L1. Thus, pembrolizumab showed anti-tumor activity and disease control with acceptable safety in patients with docetaxel-refractory mCRPC, regardless of PD-L1 status. Of note, the response rate was numerically higher in patients with somatic *BRCA1/BRCA2* or *ATM* mutations (12%), indicating that these could be predictive markers of response to checkpoint inhibitors. It can be seen from Table 2.5[61] that testing of *BRCA1/BRCA2* is NCCN recommended. Testing of *ATM* is also suggested but not yet NCCN recommended.

Gynecologic cancers

Endometrial cancers. As noted above (see Microsatellite instability high tumors and DNA mismatch repair), the presence or absence of MSI should be determined through universal tumor molecular testing in every patient with uterine cancer (see Table 2.6).[62] Approximately 2% to 5% of uterine cancers are because of Lynch syndrome, caused by germline mutations in *MLH1*, *MSH2*, *MSH6*, or *PMS2*. Abnormalities in *MLH1* should prompt hypermethylation testing, as this can also cause tumors to be MSI-H in the absence of a germline mutation. The detection of a germline mutation affects subsequent screening for colon and ovarian cancer and prompts cascade testing to identify other affected family members. The presence of MSI-H because of either a germline mutation or hypermethylation provides an indication for pembrolizumab in the setting of recurrent uterine cancer, based on site-agnostic FDA approval granted in 2017.[13] Women with *POLE*-aberrant endometrial cancers demonstrate a favorable prognosis and may require less aggressive therapy, although this remains theoretical at present. Identification of hotspot mutations in genes such as *BRAF*, *KRAS*, *PIK3CA*, and *PTEN* may correlate with biological behavior but are not yet targetable.

TABLE 2.5. Currently Recommended Predictive Molecular Testing for Prostate Cancers

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
BRCA1 and *BRCA2*	Mutation (somatic and germline)	Initial workup: If the patient has a strong family history on initial diagnosis[a] If the patient has metastatic, castration-resistant disease	NGS	A known germline mutation could help guide therapy (eg, PARP and other DDR enzyme inhibitors)	Lower level; wide acceptance	Prostate cancers
ATM	Germline mutation	Initial workup showing strong family history If patient has metastatic castration-resistant disease	NGS	NCCN guidelines recommend inquiring about known *BRCA1/BRCA2* mutations in a patient's family for prostate cancer early detection[61] and Na et al[63] proposed that, if a patient's family member died of prostate cancer before age 75 y, a genetic test of *BRCA1BRCA*/2 and *ATM* is recommended Known *BRCA1*/*BRCA2* and *ATM* germline mutations could help guide therapy with PARP and other DNA damage–response enzyme inhibitors	Lower level[b]	Prostate cancers

Abbreviations: DDR, DNA damage repair; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; PARP, poly(adenosine diphosphate-ribose) polymerase.
[a]Having at least one close relative with prostate cancer (possibility of germline mutations) and/or at least one close relative with breast, ovarian, or pancreatic cancer (possibility of a *BRCA2* germline mutation) or with colorectal, endometrial, gastric, ovarian, pancreatic, small bowel, urothelial, kidney, or bile duct cancer (possibility of Lynch syndrome through germline mutations in *MLH1*, *MSH2*, *MSH6*, or *PMS2*) is a risk-factor.
[b]Although *ATM* testing is not yet recommended by the NCCN as a predictive measure, Na et al[63] showed that germline *BRCA2* and *ATM* mutations distinguish lethal from indolent prostate cancers and are associated with shorter survival times and earlier age at death. Antonarakis et al[58] reported that patients with metastatic, castration-resistant prostate cancer harboring germline mutations in *BRCA1/BRCA2* and *ATM* have superior clinical outcomes after first-line treatment with abiraterone and enzalutamide.

TABLE 2.6. Currently Recommended Predictive Molecular Testing for Endometrial Cancers

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
ESR1 (ER)	Expression	In the stage III, IV, and recurrent disease settings	IHC	ER positivity predicts response to endocrine therapy	Lower level, wide acceptance	Uterine neoplasms, endometrial carcinoma
PMS2 (Lynch syndrome, MMR gene)	Expression	Upon diagnosis or upon recurrence if not previously tested	IHC	Loss of *PMS2* positivity indicates MMR, possible Lynch syndrome, and susceptibility to checkpoint inhibitors	Recommended by SGO Clinical Practice Statement	Uterine neoplasms, endometrial carcinoma

Abbreviations: ER, estrogen receptor; IHC, immunohistochemistry; MMR, mismatch repair; SGO, Society of Gynecologic Oncology.

Phase 2 data demonstrate activity of mTOR inhibitors in endometrioid carcinoma of the uterus, but these trials were not assay-directed to determine whether molecular testing can select for potential activity.[64]

Ovarian cancers. The presence of pathogenic mutations in *BRCA*-related genes identify an important subset of high-grade serous epithelial ovarian cancers that have a specific biology, natural history, and susceptibility to platinum and PARP inhibitors. The spectrum of mutations in this category includes those in *BRCA1*, *BRCA2*, *RAD51C*, *RAD51D*, *BARD1*, *BRIP1*, *PALB2*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *STK11* (see Table 2.7).[65-67] Patients with these mutations have an improved prognosis with a higher likelihood of platinum sensitivity and long-term survival. Homologous recombination (HR)–deficient (HRD) tumors act similarly to tumors that have *BRCA*-related mutations and may serve as a surrogate for platinum sensitivity. Identification of these mutations directly affects therapy, as patients should be considered for treatment with PARP inhibitors immediately after upfront therapy with platinum and a taxane, based on the improved progression-free survival (PFS) observed in the SOLO-1 trial (Olaparib Maintenance Monotherapy in Patients

With BRCA Mutated Ovarian Cancer Following First Line Platinum Based Chemotherapy; ClinicalTrials.gov identifier NCT01844986).[68] This international superiority trial showed a 70% reduction in risk of ovarian cancer progression in women with *BRCA* germline or somatic mutations who received maintenance olaparib after primary therapy with paclitaxel and carboplatin. Conversely, patients without *BRCA*-related mutations may be better served by antiangiogenic therapy with bevacizumab concurrent with upfront platinum and taxane therapy followed by maintenance bevacizumab therapy (Gynecologic Oncology Group study 0218 [GOG-7]).[69,70]

In the recurrent setting, PARP inhibitors (olaparib and rucaparib) as monotherapy were first approved for ovarian cancer patients with *BRCA1/BRCA2* mutations or HRD. This indication has now been expanded to include olaparib, rucaparib, and niraparib as switch maintenance therapy for patients with platinum-sensitive ovarian cancer who have responded to platinum in the second-line or third-line setting.[65-67,71]

The identification of *BRCA*-related gene mutations is also necessary to perform cascade testing on family members to identify affected family members who may be candidates for risk-reducing surgery and surveillance to prevent subsequent ovarian, tubal, peritoneal, and breast cancer.

Evaluation of PD-1 and PD-L1 status is useful in patients with ovarian cancer because pembrolizumab is approved for patients with MSI-H tumors based on a site-agnostic label. Single-agent activity for PD-1 inhibitors has been limited in patients with ovarian cancer, but checkpoint inhibitors are under study in the JAVELIN trials. The combination of PARP inhibitors with checkpoint inhibitors has been investigated, and initial response rates of 25% to 30% have been noted. The larger ATHENA trial (A Study in Ovarian Cancer Patients Evaluating Rucaparib and Nivolumab as Maintenance Treatment Following Response to Front-Line Platinum-Based Chemotherapy; ClinicalTrials.gov identifier NCT03522246) of maintenance rucaparib and nivolumab therapy is currently accruing patients with ovarian cancer who have responded to front-line, platinum-based chemotherapy.

Although initial trial results using MEK inhibitors in the treatment of patients with low-grade serous carcinomas have been disappointing, multiple studies are ongoing investigating MEK inhibitor monotherapy and combination therapy. Other rare ovarian cancers have different molecular profiles, but targeted therapies remain largely unstudied.

Cervical cancers. The treatment of patients with recurrent cervical cancer has been problematic, and their prognosis is dismal. Bevacizumab was approved for recurrent disease in combination with platinums, taxanes, and topotecan; however, no molecular markers have yet been found that can predict patient treatment response. Pembrolizumab was FDA-approved in 2018 for patients with recurrent and metastatic cervical cancer who had disease progression on or after chemotherapy and whose tumors expressed PD-L1, based on a 14% objective response rate seen in KEYNOTE 158 (Study of Pembrolizumab [MK-3475] in Participants With Advanced Solid Tumors; ClinicalTrials.gov identifier NCT02628067). Promising data also exist for single-agent nivolumab, which demonstrates a 26% response rate in the recurrent setting (ClinicalTrials.gov identifier NCT02488759). Trials evaluating combination therapy with nivolumab and ipilimumab are currently underway.

Breast cancers

The well-established biomarkers that drive treatment decisions for patients with breast cancers are estrogen receptor (ER) expression, progesterone receptor (PR) expression, and human epidermal growth factor receptor-2 (HER2) overexpression or amplification in the tumor (see Table 2.8). Determination of ER, PR, and HER2 status is recommended for all newly diagnosed invasive breast cancers and for any recurrences when feasible. These are routinely used to predict response to therapy and guide treatment planning for patients with breast cancer.

Some new markers that show promise for future use in breast cancer are the androgen receptor (AR), *ESR1*, and PD-L1. Overexpression of AR occurs in a subset of triple-negative breast cancers (TNBC).[72] Clinical trials of AR-targeted treatments have shown promising preliminary results in patients with metastatic, AR-positive TNBC.[73] Mutations in *ESR1* occur in the ligand-binding domain of the ER and can lead to a ligand-independent, constitutively active form of the ER. This is a potential mechanism of resistance to aromatase inhibitors. De novo *ESR1* mutations have been most commonly detected during or after treatment with aromatase inhibitors for hormone receptor-positive breast cancer.[74] The treatment implication is to consider using selective ER downregulators that target ER directly in the setting of an *ESR1* mutation. The role of PD-L1 as a predictive biomarker for the treatment of patients with breast cancer using checkpoint inhibitors will be further delineated with several maturing trials evaluating immune checkpoint blockade in the treatment of breast cancer. In addition, multiparameter genomic assays, such as Oncotype DX (Table 2.8), MammaPrint, and Prosigna (formerly called PAM 50), are being used routinely for decision making in early-stage breast cancer. MammaPrint and Prosigna are prognostic for recurrence of tumors that are lymph node negative, have 1 to 3 positive lymph nodes, or are ER-positive but HER2-negative. Additional multigene assays used for consideration of adjuvant therapy in patients with breast cancer are EndoPredict and the Breast Cancer Index.

TABLE 2.7. Currently Recommended Predictive Molecular Testing for Ovarian Cancers[a]

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
BRCA1 and *BRCA2*	Mutation	Recurrent disease; initial workup if the patient has a strong family history on initial diagnosis	NGS	Include other homologous recombination pathway genes and MSI or DNA MMR; helps guide therapy (eg, PARP or other DDR enzyme inhibitors; chemotherapy response)	Lower level, wide acceptance	Ovarian cancer
ATM	Mutation	Recurrent disease; initial workup if the patient has a strong family history on initial diagnosis	NGS	Include other homologous recombination pathway genes and MSI or DNA MMR; helps guide therapy (eg, PARP or other DDR enzyme inhibitors; chemotherapy response)	Lower level, wide acceptance	Ovarian cancer
BRiP1	Mutation	Recurrent disease; initial workup if the patient has a strong family history on initial diagnosis	NGS	Include other homologous recombination pathway genes and MSI or DNA MMR; helps guide therapy (eg, PARP or other DDR enzyme inhibitors; chemotherapy response)	Lower level, wide acceptance	Ovarian cancer
CHEK2	Mutation	Recurrent disease; initial workup if the patient has a strong family history on initial diagnosis	NGS	Include other homologous recombination pathway genes and MSI or DNA MMR; helps guide therapy (eg, PARP or other DDR enzyme inhibitors; chemotherapy response)	Lower level, wide acceptance	Ovarian cancer
PALB2	Mutation	Recurrent disease; initial workup if the patient has a strong family history on initial diagnosis	NGS	Include other homologous recombination pathway genes and MSI or DNA MMR; helps guide therapy (eg, PARP or other DDR enzyme inhibitors; chemotherapy response)	Lower level, wide acceptance	Ovarian cancer
RAD51C, RAD51D	Mutation	Recurrent disease; initial workup if the patient has a strong family history on initial diagnosis	NGS	Include other homologous recombination pathway genes and MSI or DNA MMR; helps guide therapy (eg, PARP or other DDR enzyme inhibitors; chemotherapy response)	Lower level, wide acceptance	Ovarian cancer

Abbreviations: DDR, DNA damage repair; MMR, mismatch repair; MSI, microsatellite instability; NGS, next-generation sequencing; PARP, poly(adenosine diphosphate-ribose) polymerase.
[a]Approximately 25% of ovarian cancers have germline or somatic mutations in *BRCA*-related or *BRCA*-related genes, including *BRCA1*, *BRCA2*, *PALB2*, *BRIP1*, *RAD51C*, and *RAD51D*. These tumors are targetable by PARP inhibitors, but the US Food and Drug Administration recently extended the approval of the PARP inhibitors niraparib, rucaparib, and olaparib to patients who do not express these mutations.[65-67]

TABLE 2.8. Currently Recommended Predictive Molecular Testing for Breast Cancers

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
ER	Expression	Noninvasive, invasive, early stage, metastatic	IHC	Predictor of response to endocrine therapy	High-level, wide acceptance	Noninvasive and invasive breast cancer, stage I-IV
PR	Expression	Invasive, early stage, metastatic	IHC	Predictor of response to endocrine therapy	High-level, wide acceptance	Invasive breast cancer, stage I-IV
HER2	Gene amplification	Invasive, early stage, metastatic	ISH	Predictor of response to HER2-targeted therapy such as trastuzumab, pertuzumab, lapatinib, or trastuzumab emtansine	High-level, wide acceptance	Invasive breast cancer, stage I-IV
HER2 (ERBB2)	Protein expression	Invasive, early stage, metastatic	IHC	Predictor of response to HER2-targeted therapy such as trastuzumab, pertuzumab, lapatinib, or trastuzumab emtansine	High-level, wide acceptance	Invasive breast cancer, stage I-IV
BRCA1 and *BRCA2*	Germline mutation	Metastatic[a]	NGS	Predictor of response to PARP inhibitor	High-level, wide acceptance	Invasive breast cancer, stage IV
Oncotype Dx	Gene expression	Hormone receptor-positive, HER2-negative	RT-PCR	Prognostic for recurrence in lymph node–negative ER-positive/HER2-negative; predictive of chemotherapy benefit in lymph node–negative ER-positive/HER2-negative	High-level, wide acceptance	Stage I, II ER-positive/PR-positive, HER2-negative

Abbreviations: ER, estrogen receptor; IHC, immunohistochemistry; NGS, next-generation sequencing; ISH, in situ hybridization; PARP, poly (ADP-ribose) polymerase; PR, progesterone receptor; RT-PCR, reverse transcription polymerase chain reaction.
[a]*BRCA* status can be assessed in early-stage disease (see National Comprehensive Cancer Network guidelines), but PARP inhibitors are not administered in this setting. For genetic/familial high-risk assessment (breast and ovarian), the following mutations are assessed in a gene panel (these markers are primarily homologous repair deficiency-related):

BRCA1/2	*TP53*	*ATM*	*NBN*	*RAD51C*	*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM* deletion
MUC16	*SKT11*	*BRIP1*	*NF1*	*RAD51D*	*MLH1*, *MLH2*, *MSH6*, *PMS2*
PTEN	*CDH1*	*CHEK2*	*PALB2*	*CDK4/6*	*EPCAM* (*TACSTD1*) deletion

Central nervous system cancers

Although broad panels are often appropriate and especially meaningful in the metastatic setting when conventional therapy has failed, more limited panels may be a consideration (see Table 2.9[75,76]). This can be exemplified by central nervous system tumors, in which genetic alterations are not just prognostic or predictive, but diagnostic. Before 2016, the World Health Organization (WHO) classification relied strictly on histologic features to differentiate tumors of astrocytic and oligodendroglial lineage.[77] Although patients have significantly different treatment paradigms and survival depending on which of these tumor lineages they harbor,[78] occasionally features from both lineages can be found within the same tumor, resulting in a diagnosis of a "hybrid" oligoastrocytoma. This is further compounded by high interobserver discordance; thus, some institutions diagnose this entity more frequently than others.[79] By combining both genotype and classical histologic findings, it is now possible to diagnose nearly all of these tumors to be compatible with either oligodendroglioma or astrocytoma. This has resulted in modifications to the WHO classification in 2016 to include both histologic phenotype and molecular genotype with consideration of *IDH* mutation, 1p19q codeletion, *ATRX* loss, and *TP53* mutation when diagnosing gliomas.[75] Furthermore, epigenetic silencing of the promoter of the methyl-guanine methyl transferase gene *MGMT* by gene promoter methylation is frequently tested because it is highly prognostic and also predictive, correlating with a response to or benefit of alkylating agent chemotherapy.[76]

It has been found that most glioblastomas have potential actionable genomic alterations.[80,81] A recent NGS analysis using a 315-gene panel found that, of 43 patients, 95% had at least 1 therapeutically actionable genomic alteration of a median of 4.5 genomic alterations per patient. The most common genomic alteration detected was in *EGFR* (40%). Genotype-directed treatments were prescribed in 13 patients, representing a 30% treatment decision impact. Treatment with targeted agents—including everolimus as a single agent and in combination with erlotinib,

TABLE 2.9. Currently Recommended Predictive Molecular Testing for Central Nervous System Cancers

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
1p19q Co-deletion[75]	Chromosome deletion	Preadjuvant therapy	FISH, PCR	Also prognostic and diagnostic: helps confirm the oligodendroglial character of tumors with ambiguous histologic features; the 1p19q co-deletion provides a good prognosis and predicts response to alkylating chemotherapy alone and in combination with radiation	Lower level, limited acceptance	Adult, low-grade, infiltrative, supratentorial astrocytoma/oligodendroglioma; anaplastic gliomas/glioblastoma
IDH1, *IDH2*	Mutation	Preadjuvant therapy glioma workup	NGS, IHC	Also prognostic and diagnostic: *IDH1* and *IDH2* mutations are related with a favorable prognosis and help in clinical trials; infiltrative gliomas that are wild-type *IDH1* or *IDH2* are likely aggressive tumors; *IDH1* or *IDH2* mutations are associated with survival benefit when treated with radiation or alkylator chemotherapy and are commonly associated with *MGMT* promoter methylation	Lower level, limited acceptance	Adult, low-grade, infiltrative, supratentorial astrocytoma/oligodendroglioma; anaplastic gliomas/glioblastoma
MGMT[76]	Promoter methylation	Preadjuvant therapy	Methylation-specific PCR, pyrosequencing	Also prognostic: strongly associated with *IDH*[75] status and genome-wide epigenetic changes (G-CIMP); translates into a survival advantage in glioblastoma, even in *IDH* wild-type tumors; used for risk stratification in clinical trials; used in treatment decisions for elderly patients with high-grade gliomas (grades III-IV); any patient with an *MGMT* promoter-methylated glioblastoma obtains greater benefit from treatment with temozolomide than patients without *MGMT* promoter methylation	Lower level, limited acceptance	Adult, low-grade, infiltrative, supratentorial astrocytoma/oligodendroglioma; anaplastic gliomas/glioblastoma

Abbreviations; FISH, fluorescence in situ hybridization; G-CIMP, glioma CPG island methylator phenotype; IHC, immunohistochemistry; NGS, next-generation sequencing; PCR, polymerase chain reaction.

afatinib, palbociclib, trametinib, and BGJ398—elicited no response.[82]

A fusion between Brevican (*BCAN*) and *NTRK1* is a potent oncogenic driver of high-grade gliomas and confers sensitivity to entrectinib.[83] A case report of a *BCAN-NTRK1* fusion in glioneuronal tumors highlights its clinical importance as a novel, targetable alteration,[84] and an open-label, multicenter, global phase 2 basket study of entrectinib for the treatment of patients with locally advanced or metastatic solid tumors that harbor *NTRK1/NTRK2/NTRK3*, *ROS1*, or *ALK* rearrangements (ClinicalTrials.gov identifier NCT02568267) is currently recruiting glioma patients.

For pediatric low-grade gliomas, *BRAF* V600E is a potentially highly targetable tumor mutation, which was detected in 17% of patients who exhibited poor outcome on receipt of chemotherapy treatment.[85] In a recent evaluation of dabrafenib in a phase 1/2 trial that included 32 children with relapsed or refractory, low-grade gliomas, findings of an objective response rate of 38% and stable disease in another 44% of patients are extremely exciting. It is encouraging that these drugs could be effective agents that allow us to replace chemotherapy entirely for pediatric glioma.[86]

Other central nervous system types for which molecular profiling has a role include ependymoma (*RELA* fusion), diffuse midline cerebellar gliomas (histone 3 mutations), medulloblastoma (*WNT* vs *SHH* activated), and ependymoma (*C19MC* amplification). Although many of these tumors inevitably recur and a broader panel may be useful at some point in the course of the disease to define clinical trial options, obtaining a limited panel that contains the molecular alterations considered within the WHO criteria remains a reasonable option.

We certainly see the potential implication of molecular profiling for a routine part of therapeutic decision making beyond classification and prognostic prediction for patients with glioma. Of other mutations tested, the epidermal growth factor gene *EGFR* variant vIII encodes a promising molecular target. *EGFR* amplification could be useful in the treatment of glioblastomas. However, agents targeting EGFR signaling pathways have displayed limited or no therapeutic efficacy in glioblastoma clinical trials. ABT-414 (an investigational, anti-EGFR monoclonal antibody drug conjugate) alone[87] or in combination with temozolomide showed a trend toward improved survival and was safely administered with radiation therapy.[88,89]

The *BRAF* V600E mutation, which is analyzed using NGS, is predictive and prognostic for low-grade pediatric glioma. This mutation is frequently found in gangliogliomas and in about two-thirds of grade II xanthroastrocytomas. It is assumed that this alteration constitutively activates the RAS/RAK/MEK/ERK kinase pathway. When BRAF kinase inhibitor treatment effects are validated within low-grade glioma, the drug could transform the *BRAF* V600E

mutation from a diagnostic marker to predictive marker of response to therapy.[90]

Sarcomas

Sarcomas are heterogeneous cancers comprising over 50 diverse histological subtypes (see Table 2.10). As a group, they have a low occurrence incidence and are considered rare cancers. Although there is some crossover, pediatric and adult sarcomas have distinctly different histologies as well as different genetic drivers. The majority of genomic variations (translocations, CNVs, complex karyotypes, etc) provide important predictive diagnostic information rather than potential therapeutic targets. Thus, *EWSR1-FLI1* (for Ewing sarcoma), *PAX3/PAX7-FOX01* (alveolar rhabdomyosarcoma), *SYT-SSX2* (monophasic synovial sarcoma), *SYT-SSX1/SSX2* (biphasic synovial sarcoma), and *TLS-FUS/CHOP* (myxoid liposarcoma) fusions are diagnostic markers that should be tested at initial workup using RNA sequencing techniques, particularly FISH. There are several prominent exceptions to this diagnosis-only rule in gastrointestinal (GI) stromal tumors, in which mutations in *KIT* (particularly exon 11) and *PDGFRA* are notable biomarkers for therapeutic intervention with the tyrosine kinase inhibitors imatinib and sunitinib.

Head and neck cancers

PD-1 is highly expressed in head and neck squamous cell carcinomas (HNSCCs) and, in 2016, the PD-1 inhibitors nivolumab[91] and pembrolizumab[92] were both approved for the treatment of HNSCC that has metastasized or recurred on or after treatment with platinum chemotherapy (see Table 2.11). PD-L1 testing is now recommended in patients undergoing workup for metastatic HNSCC, with the intention of offering pembrolizumab as a treatment option to those with PD-L1–positive tumors.

EGFR is reportedly overexpressed in between 90% and 100% of HNSCCs.[93] Accordingly, cetuximab is an approved targeted therapy for this disease and is usually administered regardless of *EGFR* mutation testing. HNSCCs can develop resistance to cetuximab. Activation of other EGFR family members (HER2, HER3) can play a role in this resistance, as can c-MET, insulin growth factor receptor (IGFR), and PI3K.[94] *PIK3CA* is frequently mutated in HNSCC and plays a key role in the progression of HNSCC.[95,96] Targeted agents against all these markers have been developed and have undergone or are undergoing various phases of clinical testing. Human papillomavirus (HPV)-related HNSCCs are increasing in incidence and have different oncogenic processes compared with HPV-unrelated HNSCCs. Patients with HPV-positive HNSCCs respond better to treatment and have a better prognosis than their HPV-negative counterparts. Therefore, for the sake of disease diagnosis, treatment, and management, it is useful to accurately discriminate between the HPV-positive and HPV-negative HNSCCs, which can be done through tumor P16 testing by IHC; thus, P16 positivity corresponds to HPV positivity.[97]

Melanomas

To date, the only FDA-approved predictive biomarker in patients with advanced melanoma is *BRAF* genotyping (see Table 2.12). Approximately one-half of melanomas that originate from cutaneous primary sites will harbor a *BRAF* V600 mutation.[98] This leads to constitutive activation of the MAPK pathway and increased cell proliferation, metastasis, and survival mechanisms. Vemurafenib, dabrafenib, and encorafenib are BRAF-targeted therapies that preferentially inhibit cells harboring the *BRAF* V600 mutation. It is important to be aware that selective

TABLE 2.10. Currently Recommended Predictive Molecular Testing for Sarcomas

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
MDM2, CDK4[a]	Amplification	At diagnosis	NGS	Possible clinical trial with CDK4/CDK6 inhibitor	Wide acceptance[a]	Well-differentiated liposarcoma, dedifferentiated liposarcoma
IDH1/IDH2[a]	Mutation	At diagnosis	NGS	Possible trial with IDH1 inhibitor	Wide acceptance[a]	Chondrosarcoma

Abbreviation: NGS, next-generation sequencing.
[a]These tests are not strictly specified in the National Comprehensive Cancer Network guidelines but are widely accepted among the sarcoma community.

TABLE 2.11. Currently Recommended Predictive Molecular Testing for Head and Neck Cancers

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
PD-L1	Protein expression	Metastatic workup	IHC	Recurrent, unresectable, or metastatic (with no surgery or radiation therapy option); second-line or subsequent therapy options: pembrolizumab for PD-L1–positive disease	Lower level, wide acceptance	Cancer of the nasopharynx

Abbreviation: IHC, immunohistochemistry; PD-L1, programmed death 1 ligand.

inhibitors of BRAF encoded by mutant *BRAF* V600 can cause paradoxical activation of the MAPK pathway in cells that are *BRAF* V600 wild-type (particularly if they harbor a *RAS* mutation). This effect occurs through RAF dimerization, leading to increased cell proliferation rather than inhibition.[99] The combination of selective BRAF inhibitors with MEK1/MEK2 inhibitors is now FDA approved only for patients with *BRAF* V600-mutant melanoma. In patients with resected, stage III, *BRAF* V600E/V600K–mutant melanoma, dabrafenib plus trametinib improves relapse-free survival by 53%.[100] Similarly, dabrafenib plus trametinib and other BRAF/MEK inhibitor combinations have demonstrated objective response rates of up to 68% in patients with unresectable advanced *BRAF* V600E/K mutant melanoma.[101]

Other oncogenic driver mutations have been identified in melanomas for which targeted therapies have demonstrated clinical activity. *KIT* mutations (and amplifications) have been identified in up to 20% of patients with advanced melanoma, particular those with chronic sun-damaged, acral, or mucosal melanoma subtypes.[102,103] Of note, *KIT* mutations are often seen across multiple exons, and hotspot mutations are not typically observed. This patient population has a reported response rate to imatinib of 21% to 29%.[104-106] Higher response rates were seen in individuals whose melanoma harbored *KIT* exon 11 and 13 mutations. Another important oncogene, *NRAS*, is mutated in approximately 20% of melanomas—most commonly at the Q61 position.[107] Direct targeting of NRAS has proven difficult, but clinical activity has been demonstrated by targeting the downstream MAPK pathway with MEK1/MEK2 inhibitors. The MEK inhibitor binimetinib showed superior clinical outcomes compared with dacarbazine.[108] However, the objective response rate of binimetinib was only 15%, and this agent has not yet been approved by the FDA for this indication.

With regard to predictive biomarkers for immune checkpoint therapies in melanoma, several have shown enrichment for greater clinical activity, mostly in post hoc or retrospective analyses, but have not been approved by the FDA for routine clinical use.[109] These include positive PD-L1 IHC, immune gene expression profiles, and high tumor mutational burden (TMB) (see The role of TMB—an emerging biomarker, below) by targeted exome sequencing. For example, response rates to anti-PD-1/PD-L1 therapy were 81%, 36%, and 10% for patients whose tumors had >23.1 mutations per megabase (MB), 3.3 to 23.1 mutations per MB, and <3.3 mutations per MB, respectively.[110] However, patients with low or negative biomarkers can still benefit from immune checkpoint therapies, and some studies have shown marginal differences between groups. The PD-L1 IHC analyses from the Checkmate 067 study demonstrate this concept well: response rates were 43% and 58% with nivolumab monotherapy in patients whose

TABLE 2.12. Currently Recommended Molecular Testing for Melanomas: Predictive and Risk Assessment Biomarkers

BIOMARKER	TEST DETECTS	WHEN	TECHNOLOGY	RECOMMENDATIONS	EVIDENCE	CANCER TYPE
BRAF	Gene mutation, V600E	Workup any time	NGS, pyrosequencing, AS-PCR	Not recommended for patients with cutaneous melanoma who otherwise have no evidence of disease (except to guide therapy)	Lower level, wide acceptance; PREDICTIVE	Melanoma
	Protein expression		IHC			
	Gene mutation	Workup for metastatic or recurrent disease	NGS, pyrosequencing, AS-PCR	Ascertain alterations in *BRAF* and *KIT* from either biopsy of the metastases (preferred) or archival material if targeted therapy is under consideration	Lower level, wide acceptance; PREDICTIVE	Melanoma
	Protein expression		IHC			
KIT	Mutation	Workup for metastatic or recurrent disease	NGS	Ascertain alterations in *BRAF* and *KIT* from either biopsy of the metastases (preferred) or archival material if targeted therapy is under consideration	Lower level, wide acceptance; PREDICTIVE	Melanoma
CDKN2A	Mutation	Follow-up; risk assessment (predisposing mutation)	NGS	If the individual has personal or familial incidence of 3 or more cases of invasive melanoma or a mix of invasive melanoma, pancreatic cancer, and/or astrocytoma, consider testing with other genes that can harbor melanoma-predisposing mutations (eg, *CDK4*, *TERT*, *MITF*, and *BAP1*)	Lower level, wide acceptance; RISK ASSESSMENT	Melanoma

Abbreviations: AS-PCR, allele-specific polymerase chain reaction; IHC, immunohistochemistry; NGS, next-generation sequencing.

TABLE 3. Frequency of MSI-H Status Across Cancer Types[a]

CANCER TYPE	% MSI-H (NO./TOTAL NO.)	
	VANDERWALDE 2018[10]	BONNEVILLE 2017[11]
All cancer types	3.0 (342/11,348)	3.8 (425/11,139)
NSCLC (adenocarcinoma/squamous cell carcinoma[b])	0.6 (12/1868)	0.5-0.6 (6/1065[b])
Colorectal adenocarcinoma	5.7 (80/1395)	–
Colon adenocarcinoma	–	19.7 (85/431)
Rectal adenocarcinoma	–	5.73 (9/157)
Pancreatic adenocarcinoma	1.2 (6/518)	0.0 (0/183)
Esophageal and esophagogastric junction carcinoma	0.0 (0/189)	1.6 (3/184)
Gastric adenocarcinoma	8.7 (16/184)	19.1 (84/440)
Liver hepatocellular carcinoma	2.7 (2/73)	0.8 (3/375)
Gastrointestinal stromal tumors (GIST)	0.0 (0/52)	–
Ovarian surface epithelial carcinoma (serous cystadenocarcinoma[c])	1.1 (17/1517)	1.37 (6/437[c])
Nonepithelial ovarian cancer	1.8 (1/56)	–
Endometrial carcinoma	17.6 (155/879)	31.4 (170/542)
Cervical cancer (squamous cell carcinoma/endocervical adenocarcinoma[d])	3.6 (6/168)	2.6 (8/305[d])
Breast carcinoma	0.6 (6/1024)	1.5 (16/1044)
Prostatic adenocarcinoma	2.1 (4/191)	0.6 (3/498)
Bladder cancer	0.0 (0/143)	0.5 (2/412)
Glioblastoma (multiforme)	0.7 (3/427)	0.3 (1/396)
(Skin cutaneous) melanoma	0.0 (0/345)	0.6 (3/470)
Head and neck squamous carcinoma	0.0 (0/111)	0.8 (4/510)
Sarcoma	–	0.78 (2/255)

Abbreviations: MSI-H, microsatellite instability-high; NO./TOTAL NO., number of MSI-H tumors/total number of tumor samples tested; NSCLC, non–small cell lung cancer.
[a]Data from: Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. *Cancer Med.* 2018;7:746-756[10]; and Bonneville R, Krook MA, Kautto EA, et al. Landscape of microsatellite instability across 39 cancer types. *JCO Precis Oncol.* 2017;1:1-15. doi:10.1200/PO.17.00073.[11]
[b]Bonneville 2017 quoted testing lung adenocarcinoma and squamous cell carcinoma, which are subtypes of NSCLC. Vanderwalde 2018 quoted general NSCLC.
[c]Bonneville 2017 quoted testing serous cystadenocarcinoma, which is a subtype of ovarian surface epithelial carcinoma. Vanderwalde 2018 quoted general ovarian surface epithelial carcinoma.
[d]Bonneville 2017 quoted testing squamous cell carcinoma and endocervical adenocarcinoma, which are subtypes of cervical cancer. Vanderwalde 2018 quoted general cervical cancer.

tumors had <5% PD-L1 staining versus >5% PD-L1 staining, respectively.[111] Biomarkers may be useful in the application of nivolumab/ipilimumab combination therapy over nivolumab monotherapy for patients with melanoma based on Checkmate 067 study data. Improvement in PFS with the combination approach was best seen in patients whose tumors harbored a *BRAF* V600 mutation or had <1% PD-L1 staining (hazard ratios, 0.62 and 0.68, respectively).

The Gray Area Between Research and Clinical Practice

Core clinical markers in current use by expert molecular profiling laboratories, the frequencies of these markers in a range of tumor lineages, and assay types used for their assessment can be found in Table 6 (Caris Molecular Intelligence). Many of the markers and their assays in Table 6 are essentially still classified as belonging in a "research" category, and the NCCN has not yet recommended universal testing for these genes. Nevertheless, they have been reported as actionable and useful by a general consensus of experts in the research community. Because the use of broader gene panels and full-scale NGS is still in the gray area between research and clinical practice, it comes burdened with benefit-to-cost ratio controversies. The field is also evolving rapidly, with fluidity existing in the classification of genes as clearly, possibly, or unlikely to be relevant to treatment considerations.

Until recently, approved genetic testing involved a small group of genetic tests carried out in patients with specific cancers for a specific therapeutic purpose. Firmly established examples of mutational status being key to treatment recommendations include pan-*RAS* testing (*KRAS*, *NRAS*, and *HRAS*) in patients with CRC to direct the use of anti-EGFR therapies cetuximab and panitumumab[45] and HER2 testing in patients with breast cancer to direct the use of anti–HER2-targeted therapies, such as trastuzumab,[112] and tyrosine kinase inhibitor therapies, such as lapatinib.[113]

TABLE 4. NTRK Frequencies in Selected Cancers[a]

NTRK GENE	TUMOR TYPE	FUSION PARTNERS[a]	FREQUENCY (NO./TOTAL NO.)[b]
NTRK1, n = 7	Gliomas	*TPM3*, *BCAN*, *MEF2D*	0.3% (3/982)
	Colorectal carcinoma	*TPM3*	0.2% (2/1272)
	Cervical carcinoma	*TPM3*	1.5% (1/68)
	Lung adenocarcinoma	*TPM3*	0.0% (1/4073)
NTRK2, n = 10	Gliomas	*VCAN*, *GKAP1*, *KCTD8*, *NOS1AP*, *TBC1D2*, *SQSTM1* (n = 2), *BCR* (n = 2), *PRKAR2A*	0.9% (9/982)
	Lung adenocarcinoma	*SQSTM1*	0.0% (1/4073)
NTRK3, n = 8	Gliomas	*EML4*, *ETV6*	0.2% (2/982)
	Lung adenocarcinoma	*ETV6*	0.0% (2/4073)
	Secretory carcinoma (breast)	*ETV6*	0.1% (1/769)
	Uterine sarcoma	*SPECC1L*	0.2% (1/478)
	Cancer of unknown primary	*ETV6*	0.4% (2/227)

Abbreviations: NO./TOTAL NO., number of tumors with fusion/total number of tumor samples tested.
[a]These were fusion partners identified by Gatalica et al,[18] and this is not a comprehensive list of all currently known *NTRK* fusion partners.
[b]The frequency data presented here are in general consensus with previous studies, although they represent a much broader overview of frequency and types of *NTRK* fusions than these other studies due to the large volume of tumors studied (more than 11,000 patients were screened). Data from: Gatalica Z, Xiu J, Swensen J, Vranic S. Molecular characterization of cancers with NTRK gene fusions. *Mod Pathol*. 2019;32:147-153.2018.[18]

It took many years and a large number of trials involving many patients before pan-*RAS* and HER2 testing became a standard treatment-predictive approach. Other examples of molecular testing used in standard clinical practice are detailed above (see Disease-Specific Biomarkers). However, as genetic testing evolved into whole genome sequencing, advances in computer technology allowed small-capacity assays to evolve into automated, high-throughput assays with large-scale data collection, classification, storage, and analysis. Thus, real-time, broad gene panel testing combined with relevant patient clinical data are now providing an unprecedented wealth of information. However, the interpretation of the meaning of results is limited by the finding that relatively small pools of evidence are available to validate most markers and their paired targeted therapies. Larger studies and collaborative efforts are certainly needed to further and more widely validate these broader panel markers and gene expression profiles and to integrate them and their targeted therapies into clinical practice (see Absence of Randomized, Controlled Clinical Trials, below). The immediate goal of testing is to translate genetic findings into potentially effective therapy decisions for today's patients. Meanwhile, numerous proof-of-principle trials currently are in progress or in development. One key to accelerating the application of this knowledge is real-time national and international partnerships between cancer researchers and pharmaceutical companies to perform broad-panel profiling and elucidate targeted patient therapies. Concurrently, data pooling is mandatory using universal data-sharing capabilities to maximize the utility of these findings and generate large pools of evidence (see Data Sharing, below). Successes and failures alike will provide a more complete picture, and the result will take us steps closer to effective cancer treatment—and cures. This model is already in practice in the form of basket trials.

Oncology Basket Trials and Precision Medicine

Current oncology basket trials test therapies across a range of populations using biomarker-driven designs. Such trials choose biomarkers, which must have a clinically feasible assay, to attempt to enrich responses to a particular targeted therapy. The gathering of efficacy data across a range of populations translates to only one primary outcome endpoint, which simplifies the situation while increasing deductive power. These large-scale and small-scale, broad-panel molecular profiling trials include the National Cancer Institute's Molecular Analysis for Therapy Choice (NCI-MATCH) trial, the American Society of Clinical Oncology (ASCO) Targeted Agent and Profiling Utilization Registry (TAPUR) study, and the European Organization for Research and Treatment of Cancer–Screening Patients for Efficient Clinical Trial Access (EORTC-SPECTA) program. These studies attempt to expand the boundaries of precision medicine and build evidence supporting the use of molecularly tailored therapy.

National Cancer Institute's Molecular Analysis for Therapy Choice Trial

The novel, phase 2 NCI-MATCH (Molecular Analysis for Therapy Choice[114]) trial was initiated in August 2015 and is bringing public and private sectors together to enable access of physician researchers to investigational agents (in addition to approved agents) in an attempt to build the much sought-after evidence supporting the effectiveness of matching

TABLE 5. Best Known Somatic Mutations That Could Also Be Germline Mutations[a]

GERMLINE OR SOMATIC MUTATION	RARE GERMLINE-ASSOCIATED SYNDROME	MAIN CANCER APPLICABILITY
TP53	Li-Fraumeni	Sarcomas, and cancers of the breast and brain
MSH2, *MLH1*, *MSH6*, *PMS2*, *EPCAM*	Lynch	Cancers of the GI tract (particularly colorectal), endometrium, ovary, brain, breast, and renal pelvis
BRCA1, *BRCA2*	Hereditary breast, ovarian, prostate, and pancreatic cancers	Cancers of the breast, ovary, prostate, and pancreas
PTEN	Cowden	Cancers of the breast, endometrium, and thyroid gland
APC, *MUTYH*	Familial adenomatous polyposis	Cancers of the colon and rectum, small intestine, stomach, brain, bone, and skin
CDH1	Hereditary diffuse gastric cancer	Cancers of the stomach and breast
CDK4, *CDKN2A*	Familial atypical multiple mole melanoma	Melanoma, pancreatic adenocarcinoma, and cerebral astrocytoma
MEN1	Werner	Pancreatic endocrine cancer and pituitary gland tumors
RB1	Retinoblastoma	Eye cancer, pinealoma, osteosarcoma, melanomas, and soft-tissue sarcomas
RET	Multiple endocrine neoplasia type 2	Medullary thyroid cancer, and pheochromocytoma
VHL	Von Hippel-Lindau	Kidney cancers and multiple noncancerous tumors
STK11	Peutz-Jeghers	Cancers of the breast, colon and rectum, pancreas, and stomach and hamartomas
SDHD, *SDHB*, *SDHC*	Familial paraganglioma	Paragangliomas and pheochromocytomas
FLCN	Birt-Hoge-Dube	Chromophobe renal cell cancers
TSC1, *TSC2*	Tuberous sclerosis	Angiofibromas, angiomyolipomas, giant cell astrocytomas
NF1	Neurofibromatosis type 1	Optic gliomas and neurofibromas
NF2	Neurofibromatosis type 2	Schwannomas, meningiomas, gliomas, neurofibromas
PTCH1	Gorlin	Childhood primitive neuroectodermal tumors, skin basal cell carcinomas
BMPR1A, *SMAD4*	Juvenile polyposis	Multiple noncancerous growths in the colon

Abbreviation: GI, gastrointestinal.
[a]Table adapted from: Lartigue J. Blurring the lines between germline and somatic mutations in cancer. *Oncol Live.* 2017;18. onclive.com/publications/oncology-live/2017/vol-18-no-13/blurring-the-lines-between-germline-and-somatic-mutations-in-cancer. Accessed February 6, 2019.[32]

targeted therapy to patient molecular profiles. The primary aim of the NCI-MATCH study is to evaluate the proportion of patients with objective responses (ORs) to targeted therapies predicted to be mechanistically effective based on individual tumor genomic profiling. If the response rate to any mutation-matched therapy is at least 25%, this match will be tested in larger phase 2 trials. There are well over a thousand study locations across the United States, and pharmaceutical and biotechnology companies are providing targeted agents to enrolled patients across these sites. Patients are treated according to their profile (Table 7) and regardless of tissue origin or cancer type. New drugs of interest can be added to the "master" trial at any time. The trial is running under ClinicalTrials.gov identifier NCT02465060, where up-to-date information can be obtained.

Targeted Agent and Profiling Utilization Registry

The TAPUR study is an ongoing, nonrandomized, multicenter clinical trial that opened in 2016.[115] This trial is testing the use of drugs already approved by the FDA that target a specific tumor mutation in individuals with advanced cancer outside of the drug's approved indication. Patients with a range of solid tumors as well as lymphomas and multiple myelomas are eligible for enrollment. As with NCI-MATCH, treatment assignment in this study is based on an existing tumor mutation and not the organ from which the cancer originated. The study aim is to observe the real-world use of targeted therapies in any patient whose tumor tests positive for a selected genomic alteration that is known to be a drug target or has been shown to predict sensitivity to a drug available in this study. The primary outcome measure is the objective response rate (defined as the percentage of participants in a cohort with a complete or partial response at 8 weeks postbaseline or with stable disease at 16 weeks or later postbaseline according to RECIST (Response Evaluation Criteria in Solid Tumors) (for solid tumors), international uniform response criteria (for multiple myeloma),[116] and

TABLE 6. Caris Molecular Intelligence Core Clinical Marker Frequencies for Tumor Lineages

NSCLC				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	10	1893	0.5
TML	NGS	712	4557	15.6
PD-L1 (22c3)	IHC	2541	5658	44.9
ALK	IHC	10	4606	0.2
MET	NGS	84	4606	1.8
MET	CNV	84	4606	1.8
MET	Fusion	84	4606	1.8
ALK	ISH/fusion	10	4606	0.2
ALK	NGS	10	4606	0.2
RET	Fusion	19	4164	0.5
ROS1	Fusion	14	4186	0.3
RET	Fusion	19	4164	0.5
PTEN	IHC	7792	12,618	61.8
BRAF	NGS	165	4606	3.6
EGFR (L858R)	NGS	174	4659	3.7
EGFR (exon 19 del)	NGS	260	4659	5.6
EGFR T790M	NGS	62	4773	1.3
EGFR tertiary	NGS	3	4659	0.1
ERBB2	NGS	70	4606	1.5
KRAS	NGS	1319	4606	28.6
NTRK1/2/3	Fusion	3	3797	0.1
NTRK	IHC	35	2031	1.7
PIK3CA	NGS	219	4606	4.8
DDR2	NGS	0	4606	0.0
AKT1	NGS	9	4761	0.2

ANAL CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
TML	NGS	6	91	33.0
PD-L1	IHC	63	191	33.0
PIK3CA	NGS	22	92	33.0
TP53	NGS	10	92	33.0
MSI	NGS	0	40	0.0

PERITONEAL CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
KRAS	NGS	42	87	48.3
GNAS	NGS	24	87	27.6
SMAD4	NGS	10	87	11.5
ATM	NGS	2	87	2.3
BRAF	NGS	4	87	4.6
NRAS	NGS	1	87	1.1
PIK3CA	NGS	3	87	3.4
MLH1	IHC	190	192	99.0
PMS2	IHC	189	192	98.4
MSH2	IHC	189	192	98.4
MSH6	IHC	187	192	97.4
MSI	NGS	1	27	3.7
TML	NGS	1	87	1.1
PD-L1 (SP142)	IHC	7	211	3.3
PTEN	IHC	252	393	64.1

COLORECTAL CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
KRAS	NGS	1744	3427	50.9
NRAS	NGS	146	3427	4.3
BRAF	NGS	285	3427	8.3
PIK3CA	NGS	600	3427	17.5
POLE	NGS	14	3424	0.4
RSPO3	Fusion	32	1272	2.5
MLH1	IHC	5840	6132	95.2
PMS2	IHC	5719	6097	93.8
MSH2	IHC	6011	6121	98.2
MSH6	IHC	5947	6104	97.4
MSI	NGS	83	1346	6.2
TML	NGS	247	3400	7.3
HER2	IHC	161	8708	1.8
HER2	CISH	129	4460	2.9
PD-L1	IHC	196	6405	3.1
PTEN	IHC	5334	11,407	46.8

TABLE 6. *Continued*

SMALL CELL LUNG CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	0	88	0.0
TML	NGS	13	205	6.3
PD-L1	IHC	182	206	88.3
TP53	NGS	99	205	48.3
RB1	NGS	11	206	5.3
NOTCH1	NGS	0	206	0.0
RET	NGS	0	206	0.0
SMO	NGS	0	49	0.0

GASTROESOPHAGEAL CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
ATM	NGS	31	922	3.4
BLM	NGS	5	921	0.5
CDH1	NGS	50	921	5.4
MLH1	NGS	6	922	0.7
MSH2	NGS	3	921	0.3
MSH6	NGS	15	921	1.6
PMS2	NGS	2	921	0.2
SMAD4	NGS	44	921	4.8
BMPR1A	NGS	7	921	0.8
TP53	NGS	686	922	74.4
PTEN	NGS	30	922	3.3
APC	NGS	58	922	6.3
STK11	NGS	17	921	1.8
PD-L1	IHC	180	1804	10.0
MSI	NGS	15	389	3.9
TML	NGS	47	916	5.1
HER2	IHC	75	1431	5.2

CHOLANGIOCARCINOMA				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
IDH1	NGS	39	474	8.2
IDH2	NGS	14	474	3.0
BAP1	NGS	35	474	7.4
PBRM1	NGS	23	474	4.9
KRAS	NGS	82	474	17.3
NRAS	NGS	16	474	3.4
BRAF	NGS	13	474	2.7
PIK3CA	NGS	30	474	6.3
MLH1	IHC	70	73	95.9
PMS2	IHC	69	73	94.5
MSH2	IHC	72	73	98.6
MSH6	IHC	72	73	98.6
MSI	NGS	1	177	0.6
TML	NGS	15	469	3.2
HER2/*neu*	IHC	40	1301	3.1
HER2	CISH	38	618	6.1
PD-L1	IHC	78	934	8.4
BRAF	Fusion	2	131	0.0
FGFR3	Fusion	1	149	0.7
FGFR2	Fusion	7	149	4.7

SMALL BOWEL CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
KRAS	NGS	100	183	54.6
NRAS	NGS	3	183	1.6
BRAF	NGS	14	183	7.7
PIK3CA	NGS	26	183	14.2
POLE	NGS	1	183	0.5
MLH1	IHC	88	96	91.7
MSH2	IHC	90	96	93.8
MSH6	IHC	89	96	92.7
PMS2	IHC	89	96	92.7
MSI	NGS	10	71	14.1
TML	NGS	18	182	9.9
HER2	IHC	10	482	2.1
HER2	CISH	11	269	4.1
PD-L1	IHC	25	342	7.3
PTEN	IHC	264	526	50.2
RSPO3	Fusion	6	57	10.5

TABLE 6. *Continued*

GASTROESOPHAGEAL CANCER (CONTINUED)				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
HER2	CISH	168	1323	12.7
PIK3CA	NGS	34	462	7.4
CDKN2A	NGS	78	922	8.5

PANCREATIC CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	6	456	1.3
TML	NGS	18	1149	1.6
KRAS	NGS	942	1164	80.9
NRAS	NGS	2	1164	0.2
BRAF	NGS	18	1164	1.5
CDKN2A	NGS	229	1164	19.7
HER2	IHC	38	2096	1.8
HER2	ISH	26	4063	0.6
PD-L1	IHC	222	2682	8.3
ATM	NGS	42	1164	3.6
BRCA1	NGS	13	1031	1.3
BRCA2	NGS	36	1031	3.5

RENAL CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	1	107	0.9
TML	NGS	3	327	0.9
BAP1	NGS	33	333	9.9
FH	NGS	2	332	0.6
FLCN	NGS	1	332	0.3
MET	NGS	6	333	1.8
PBRM1	NGS	60	332	18.1
SDHD	NGS	0	332	0.0
SETD2	NGS	46	332	13.9

HEPATOCELLULAR CARCINOMA				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
CTNNB1	NGS	53	166	31.9
TP53	NGS	56	166	33.7
ARID1A	NGS	8	166	4.8
MSI	NGS	1	69	1.4
TML	NGS	4	166	2.4
PD-L1	IHC	26	366	7.1

ENDOMETRIAL CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
TML	NGS	282	1469	19.2
MSI	NGS	163	796	20.5
MSI	Frag analysis	267	1831	14.6
POLE	NGS	42	1841	2.3
PTEN	NGS	1137	1843	61.7
PIK3CA	NGS	823	1843	44.7
ARID1A	NGS	745	1841	40.5
HER2 (ERBB2)	NGS	21	1843	1.1
HER2 (ERBB2)	CNV	61	1867	3.3
MSH6	IHC	2273	2295	99
MSH2	IHC	2301	2306	99.8
PMS2*	IHC	1887	2293	82.3
MLH1*	IHC	1912	2298	83.2
PD-L1	IHC	460	4502	10.2

BLADDER CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	4	152	2.6
TML	NGS	58	361	16.1
ATM	NGS	21	364	5.8
BRCA1	NGS	12	364	3.3
BRCA2	NGS	17	364	4.7
ERCC2	NGS	0	364	0.0
FANCC	NGS	0	364	0.0
FGFR3	NGS	43	364	11.8
RB1	NGS	55	364	15.1
TSC1	NGS	23	364	6.3
PD-L1	IHC	162	788	20.6

PROSTATE CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	6	151	4.0
TML	NGS	15	416	3.6
ATM	NGS	22	422	5.2
BRCA1	NGS	6	365	1.6
BRCA2	NGS	26	365	7.1
AR	NGS	35	440	8.0
PD-L1	IHC	25	811	3.1
AR	IHC	1127	1213	92.9
TMPS:ERG2	Fusion	57	198	28.8

TABLE 6. *Continued*

RENAL CANCER (CONTINUED)				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
TSC1	NGS	5	332	1.5
TSC2	NGS	2	332	0.6
VHL	NGS	153	333	45.9
PD-L1	IHC	117	630	18.6

OVARIAN CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	23	1484	1.5
TML	NGS	63	3434	1.8
BRCA1	NGS	238	2924	8.1
BRCA2	NGS	180	2922	6.2
ATM	NGS	63	3463	1.8
PIK3CA	NGS	290	3463	8.4
PTEN	NGS	158	3463	4.6
KRAS	NGS	253	3463	7.3
NF1	NGS	170	3463	4.9
ARID1A	NGS	279	3458	8.1
SMARC4A	NGS	19	3452	0.6
ER	IHC	7947	16,384	48.5
PR	IHC	4698	16,353	28.7
PD-L1	IHC	748	8693	8.6

CERVICAL CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	2	116	1.7
TML	NGS	20	305	6.6
TP53	NGS	56	307	18.2
PIK3CA	NGS	92	307	30.0
PD-L1	IHC	191	802	23.8

BREAST CANCER				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	5	779	0.6
TML	NGS	76	2146	3.5
PD-L1	IHC	389	5519	7
AR	IHC	5698	11,114	51.3
ER	IHC	5598	9404	59.5
PR	IHC	3806	9362	40.7
HER2	IHC	900	9299	9.7
HER2	CISH	726	6087	11.9
ATM	NGS	50	2169	2.3
BRCA1	NGS	59	1960	3.0
BRCA2	NGS	88	1960	4.5
CDH1	NGS	160	2164	7.4
CHEK2	NGS	32	2169	1.5
ESR1	NGS	159	2164	7.3
FLCN	NGS	3	2164	0.1
NBN	NGS	4	2164	0.2
NF1	NGS	86	2169	4
PALB2	NGS	27	2164	1.2
PTEN	NGS	143	2169	6.6
STK11	NGS	15	2164	0.7
TP53	NGS	1203	2169	55.5

GLIOBLASTOMA				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
IDH1	NGS	161	851	18.9
IDH2	NGS	3	851	0.4
BRAF	NGS	17	851	2.0
MSI	NGS	1	265	0.4
TML	NGS	28	847	3.3
ATRX	NGS	59	851	6.9
CIC	NGS	18	851	2.1
FUBP1	NGS	8	851	0.9
MGMT	Methylation	828	1773	46.7
1p19q	FISH	52	779	6.7
*EGFR*vIII	Fusion	87	630	13.8
EGFR	CNV	257	828	31
PD-L1	IHC	209	1346	15.5
NTRK	Fusion	8	404	2.0
MET	Fusion	11	404	2.7
EGFR	Fusion	3	404	0.7
FGFR3	Fusion	12	404	3.0
BRAF	Fusion	2	404	0.5
PDGFRA	Fusion	1	404	0.2

TABLE 6. *Continued*

SARCOMA				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	6	350	1.7
TML	NGS	32	1025	3.1
RB1	NGS	63	1034	6.1
NF1	NGS	51	1036	4.9
KIT	NGS	1	601	0.2
PDGFRA	NGS	0	1036	0.0
EWSR1	Fusion	3	28	10.7
RET	Fusion	1	469	0.2
ALK	Fusion	3	470	0.6
NTRK1	Fusion	1	469	0.2
RAF1	Fusion	1	28	3.6
PD-L1	IHC	505	2386	21.2

HNSCC				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	1	164	0.6
TML	NGS	24	324	7.4
TP53	NGS	201	326	61.7
PIK3CA	NGS	43	326	13.2
PD-1	IHC	250	340	73.5
PD-L1	IHC	255	721	35.4

MELANOMA				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	0	293	0.0
TML	NGS	289	774	37.0
BRAF	NGS	313	782	40.0
KIT	NGS	20	801	2.0
MEK1	NGS	27	782	4.0
NF1	NGS	189	782	24.0
NRAS	NGS	197	782	25.0
CDKN2A	NGS	111	782	14.0
PTEN	NGS	47	782	6.0
MDM2	CNV	16	749	2.0
PD-L1	IHC	452	1381	33.0

UVEAL MELANOMA				
BIOMARKERS	TECHNOLOGY	POSITIVE NO.	TOTAL NO.	PERCENT
MSI	NGS	0	22	0.0
TML	NGS	3	97	3.1
BAP1	NGS	49	99	49.5
GNA11	NGS	43	99	43.4
GNAQ	NGS	48	99	48.5
SF3B1	NGS	11	99	11.1
PD-L1	IHC	21	139	15.1

Abbreviations: AR, androgen receptor; CISH, chromogenic in situ hybridization; CNV, copy number variation; del, deletion; ER, estrogen receptor; FISH, fluorescence in situ hybridization; Frag analysis, fragment analysis; HNSCC, head and neck squamous cell carcinoma; IHC, immunohistochemistry; ISH, in situ hybridization; MSI, microsatellite instability; NGS, next-generation sequencing; NSCLC, non–small cell lung cancer; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PR, progesterone receptor; TML, tumor mutation load.

TABLE 7. Broadening Molecular Profiling Boundaries—Biomarker-Targeted Therapy Matches

TARGETED MUTATION	DRUG
NCI-MATCH trial: NCT02465060[a]	
EGFR activating mutation	Afatinib
HER2 activating mutation	Afatinib
BRCA1 or *BRCA2* mutations	Adavosertib (AZD1775)
FGFR pathway aberrations	AZD4547
NRAS12, *NRAS13*, *NRAS61* mutation	Binimetinib
AKT mutation	Capivasertib (AZD 5363)
PIK3CA mutation	Copanlisib
PTEN mutation	Copanlisib
PTEN loss	Copanlisib
MET amplification	Crizotinib
MET exon 14 deletion	Crizotinib
ALK translocation	Crizotinib
ROS1 translocation or inversion	Crizotinib
BRAF V600E/V600R/V600K/V600D mutation	Dabrafenib + trametinib
DDR2 S768R, I638F, or L239R mutation	Dasatinib
NF2 inactivating mutation	Defactinib
PTEN mutation or deletion and PTEN expression	GSK2636771 (PI3Kβ inhibitor)
PTEN loss	GSK2636771 (PI3Kβ inhibitor)
FGFR mutation or fusion	Erdafitinib
FGFR amplification	Erdafitinib
NTRK1, *NTRK2*, *NRTK3* gene fusions	Larotrectinib (LOXO-101)
Loss of *MLH1* or *MSH2* (by IHC)	Nivolumab
EGFR T790M or rare activating mutation	Osimertinib
CCND1, *CCND2*, *CCND3* amplification & Rb expression	Palbociclib
CDK4 or *CDK6* amplification and Rb protein	Palbociclib
HER2 amplification ≥7 copy numbers	Pertuzumab + trastuzumab
TSC1 or *TSC2* mutation	Sapanisertib
mTOR mutation	Sapanisertib
cKIT exon 9, 11, 13, or 14 mutation	Sunitinib
PIK3CA mutation	Taselisib
GNAQ/*GNA11* mutation	Trametinib
BRAF fusion or *BRAF* non-V600 mutation	Trametinib
NF1 mutation	Trametinib
HER2 amplification	Trastuzumab emtansine
SMO/*PTCH1* mutation	Vismodegib
TAPUR trial: NCT02693535[b]	
VEGFR mutation, amplification or overexpression	Axitinib
Bcr-abl, *SRC*, *LYN*, *LCK* mutations	Bosutinib
ALK, *ROS1*, *MET* mutations	Crizotinib
KRAS, *NRAS*, and *BRAF* (all wild type)	Cetuximab
Bcr-abl, *SRC*, *KIT*, *PDGFRB*, *EPHA2*, *FYN*, *LCK*, *YES1* mutations	Dasatinib
BRCA1/*BRCA2* inactivating mutations; *ATM* mutations/deletions	Olaparib
MSI-high, high TML, and others	Nivolumab and ipilimumab

TABLE 7. *Continued*

TARGETED MUTATION	DRUG
CDKN2A, *CDK4*, *CDK6* amplifications	Palbociclib
POLE/*POLD1* mutations; high TML	Pembrolizumab
VEGFR1, *VEGFR2*, *VEGFR3*, *PDGFRB*, *RET*, *KIT*, *RAF-1*, *BRAF* mutations/amplifications	Regorafenib
PDGFR, *VEGFR*, *CSF1R*	Sunitinib
mTOR, *TSC* mutations	Temsirolimus
ERBB2 amplifications	Trastuzumab and pertuzumab
BRAF V600E mutations	Vemurafenib and cobimetinib

Abbreviations: IHC, immunohistochemistry; MSI, microsatellite instability; NCI, National Cancer Institute; TML, tumor mutation load.
[a]NCI-MATCH trial: Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma. Matches are as listed on clinicaltrials.gov/ct2/show/NCT02465060. Accessed February 6, 2019. After patient tumor molecular testing on a main screening protocol, those with actionable mutations are assigned to 1 of 35 treatment subprotocols.
[b]The American Society of Clinical Oncology's TAPUR trial: Testing the Use of US Food and Drug Administration-Approved Drugs That Target a Specific Abnormality in a Tumor Gene in People With Advanced Stage Cancer. Matches are as listed on clinicaltrials.gov/ct2/show/NCT02693535. Accessed February 6, 2019.

Lugano criteria (for non–Hodgkin lymphoma).[117,118] Table 7 details the genomic alterations (biomarkers) and targeted therapies of interest at the time of submission of this article for publication, although markers and therapies are continually being refined as the study progresses. It is currently anticipated that TAPUR will enroll over 2500 patients in total. The trial is running under ClinicalTrials.gov identifier NCT02693535, where up-to-date information can be obtained.

European Organization for Research and Treatment of Cancer–Screening Patients for Efficient Clinical Trial Access

EORTC-SPECTA is a collaborative European molecular screening program that coordinates several disease-specific platforms with the aim of identifying actionable mutations and offering specific targeted therapy to patients (ClinicalTrials.gov identifier NCT02834884).[119,120] This is a large-scale basket trial that operates through one entry point that provides access to multiple studies and to high-quality, annotated material for research purposes and provides longitudinal follow-up of patients to understand progression patterns.[121]

Targets of Special Interest: Emerging and in Current Practice

There are several novel biomarkers of great interest, many of which have found a niche in common practice but are continuing to reveal exciting connections and uses. We expand on several these markers below.

Immune Markers and Immunotherapy

Programmed death–ligand 1 expression

The immune checkpoint PD-1/PD-L1 axis is a well-described inhibitory pathway that leads to T-cell exhaustion in the tumor microenvironment.[122] Typically, PD-1 on tumor-infiltrating cytotoxic T cells interacts with PD-L1 on tumor cells, causing dampening of antitumor immunity (an adaptive immune response).[123] Tumor types known to be immunogenic typically have relatively high rates of PD-L1 positivity.[124] However, although greater clinical activity of anti–PD-1 agents (nivolumab and pembrolizumab) and PD-L1 agents (avelumab, atezolizumab, and durvalumab) has been consistently observed in patients with PD-L1–positive disease,[125,126] some clinical trials have found that patients with low PD-L1–expressing tumors can derive significant benefit from anti–PD-1/PD-L1 agents. Therefore, PD-L1 IHC score alone is insufficient for patient selection in many tumor types. Assays have been developed to test for PD-L1 expression, including the PD-L1 IHC assay with 28-8 Dako (developed for nivolumab), 22C3 Dako (developed for pembrolizumab), SP142 Ventana (atezolizumab), SP263 Ventana (durvalumab), and 73-10 Dako (avelumab). These assays can be used as a tool for physicians to assess which patients might have the largest chance of benefitting from anti–PD-1/PD-L1 agents. However, because each inhibitor requires its own individual PD-L1 IHC assay, it is useful to have an upfront working knowledge of which targeted therapy is going to be used; otherwise the laboratory is required to run 5 different IHC tests, which raises costs and inefficiencies. There are several scenarios in which the FDA has mandated that PD-L1 positivity is required before anti–PD-1 agents are usable within approved indications. For example, patients with advanced, metastatic NSCLC can be treated in the front line with pembrolizumab monotherapy only if their PD-L1 tumor proportion score (TPS) is >50% (Table 2.1).[127] In the second-line setting, pembrolizumab is FDA approved for adult patients with tumors (eg, gastric tumors) (Table 2.3[42]) that have a lower positive TPS score (>1%).[128] Interestingly, nivolumab has been approved as second-line therapy for select cancers regardless of their PD-L1 status.[129,130] There is controversy over the

use and reliability of PD-L1 IHC as a predictive biomarker. This is because of multiple factors:

- First, performing a PD-L1 IHC assay on a single tumor site at one time point does not take into account the intrapatient tumor heterogeneity that can exist and the variability in PD-L1 expression that can occur over time.[131] PD-L1 expression can be regulated by IFN-γ signaling from T-cell interactions and by several tumor-intrinsic pathways such as MAPK and PI3K/Akt signaling, as well as epigenetic factors.[132]
- A second issue is the range of antibody assays that have been developed[124] and the need for standardization. There have been cross assay comparisons, particularly in NSCLC, for which the staining patterns were similar among the 28-8, 22C3, and SP142 antibodies.[133,134] However, SP142 staining of tumor cell membranes was shown to be weaker, resulting in fewer positive tumor cells than some other assays. The 73-10 antibody was not included in these analyses.
- A final issue is related to PD-L1 IHC scoring: how does one define a PD-L1–positive from a PD-L1–negative tumor? PD-L1 staining of immune cells in the tumor microenvironment, such as macrophages, gives a signal that is erroneously included in tumor PD-L1 assessment. This is the case for 22C3 and SP142 assays. To date, no approaches or thresholds reach sufficient sensitivity or specificity to be predictive of a high likelihood of response to a given drug. Providers need to be familiar with the individual PD-L1 assays and scoring used for each agent and tumor type when making patient decisions based on PD-L1 results.

Microsatellite instability and deficient MMR

Microsatellites are lengths of DNA sequence that contain single nucleotide (mononucleotide) or sections of 2 or more nucleotide (dinucleotide, trinucleotide, tetranucleotide, or pentanucleotide) repeats (see Microsatellite instability-high tumors and DNA mismatch repair). When microsatellites contain a clonal change in several repeated DNA nucleotide units, this results in MSI (tumors with such MSI are characterized as MSI-H, and this occurs when at least one of the MMR genes—*MSH2*, *MLH1*, *MSH6*, and *PMS2*—are inactivated, causing dMMR).[10] Since MSI-H was established as a possible biomarker, the MSI status of a tumor has always required microdissection and PCR-based detection strategies. For practical purposes, MSI is equivalent to the loss of staining by IHC of at least one of the MMR genes because any lack of normal MMR protein expression signifies an abnormality in MMR and thus MSI. A sensitive and specific MSI assay by NGS has recently been developed that is comparable to the existing gold standard of PCR-based methods without requiring matched samples from tumor and normal tissues.[10] MSI appears to be a generalized cancer phenotype in about 4% of all adult cancers in total. MSI-H tumors are associated with an improved prognosis in early-stage cancers. In Table 3,[10,11] MSI-H frequency data for several different cancer types are compared between 2 studies. In both studies, patient DNA was originally sequenced by NGS; however, the study by Bonneville et al[11] obtained and retrospectively assessed sequencing data from The Cancer Genome Atlas (TCGA), the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) database, and 444 other studies, whereas the study by Vanderwalde et al[10] retrospectively assessed data from commercial comprehensive sequencing profiles performed on patient tumors by Caris Life Sciences. It can be readily observed from Table 3[10,11] that the rate of MSI-H in tumors from different tissue types is not always consistent between studies. In particular, this can be observed for gastric cancers, endometrial cancers, breast cancers, and CRCs versus colon and rectal cancers. These study differences could be explained for the most part by sampling bias, the use of different data analysis techniques, or statistical variance. Vanderwalde et al[10] certainly assessed a very sick patient population that was undergoing tumor profiling because of a bad prognosis and lack of obvious therapeutic options, whereas the patient population examined by Bonneville et al[11] was not described as such and possibly consisted of patients with variable disease stages and prognoses; MSI-H patients tend to have a better prognosis than their microsatellite stable counterparts do, which would explain the lower percentage of MSI-H patients in the Caris data set compared with the TCGA data set. In addition, Vanderwalde et al[10] combined colon and rectal cases in one analysis, possibly yielding a lower percentage of MSI-H than that seen by Bonneville et al[11] for colon cancer alone. This highlights the potential for variability in biomarker assessment because of different assay types and technologies, not just for MSI but also across the biomarker board.

The role of TMB—an emerging biomarker

TMB is certainly an interesting marker, and evidence of its importance is growing. However, methodologies assessing TMB are not widely available at present, and most clinical laboratories do not yet offer this assessment in their assay repertoire. Immunogenicity is certainly associated with mutation load, suggesting that an increase in the number of somatic mutations present in tumor cells increases potential recognition by the immune system.[135] Indeed, the presence of mutations in the tumor generates neoantigens (not expressed by normal cells), and the more mutations there are, the more the tumor is likely to be immunogenic. Furthermore, emerging evidence suggests

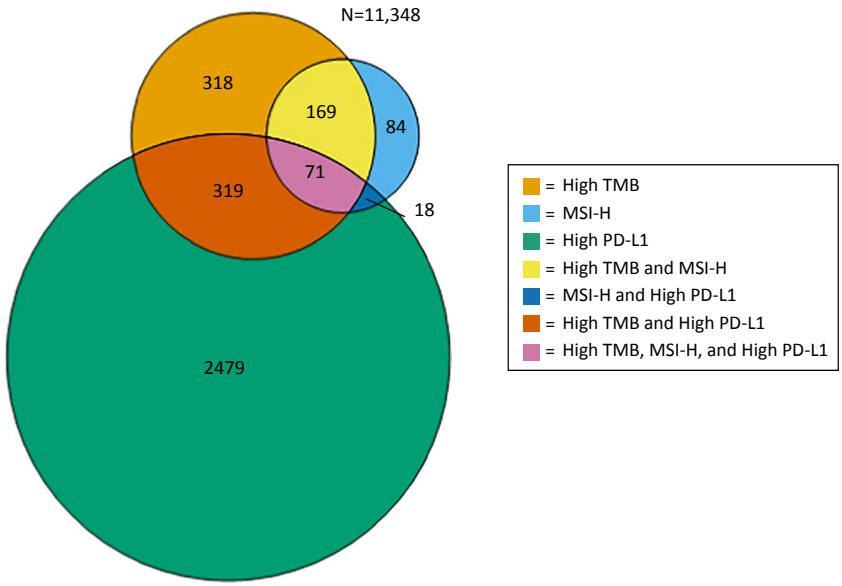

FIGURE 1. Venn Diagram of the Relationships Between High Tumor Mutational Burden (TMB), High Microsatellite Instability (MSI-H), and High Programmed Death–Ligand 1 (PD-L1) for All Cancer Types.[10]

that a high TMB is associated with increased clinical activity of immune checkpoint inhibitors.[136-140] TMB was shown to predict immune therapy efficacy in patients with melanoma,[138] NSCLC,[140] and GI cancers.[141] There is ongoing discussion regarding the definition of "high" TMB for predictive purposes. Most studies report ≥17 mutations per MB as high TMB, which is based on comparing TMB with MSI in patients with CRC. This, of course, also shows that high TMB is in strong concordance with MSI-high in CRC. However, although TMB is associated with dMMR, not all tumors with a high TMB are actually associated with MSI-H, and future studies should address this aspect.

MSI and MMR, TMB, and PD-L1

The relationship between TMB, MSI, and PD-L1 has recently been explored in a broad range of cancer types (Fig. 1).[10] There is some overlap of all 3 markers in a few cancers. However, in most cancers, overlap is infrequent or does not exist at all, and 69.5% of all cancer cases were negative for all 3 biomarkers (7890 of 11,348 tested). A population of tumors exhibiting MSI-H status but low TMB and no PD-L1 expression was identified. Since MSI/MMR status alone or in combination with PD-L1 positivity became an accepted predictive marker in the FDA indication for checkpoint inhibitors, the finding that patients can test positively for only one of these markers obviously means that the number of patients now eligible to receive and hopefully benefit from checkpoint inhibition has been broadened. Until more is understood about how MSI, PD-L1, and TMB work together and how this interaction is clinically relevant, the only reasonable option is to continue to assay for all 3 markers and ensure that the number of patients who are given the chance to benefit from these drugs is maximized.

Polybromo 1

PBRM1 is a non–MSI/PD-L1/TMB marker that could be predictive for response to checkpoint inhibitors. For example, clear cell renal cell carcinoma (ccRCC) responds to immune therapy but, unlike many other responsive human tumors, harbors a low burden of somatic mutations. Even so, ccRCC has relatively high immune cytolytic activity and a microenvironment with high immune and T-cell infiltration scores. In the past, large-scale sequencing studies demonstrated that *PBRM1* loss of function (LOF) alterations are present in a large portion (up to 41%) of ccRCC tumors. Patients whose tumors had *PBRM1* loss in both gene copies had significantly prolonged OS and PFS and manifested reduced tumor burden in response to immune checkpoint therapy compared with patients without *PBRM1* loss (log-rank $P = .0074$ and $P = .029$, respectively). Miao et al summarized that, given the high prevalence of *PBRM1* LOF in ccRCC, this genetic mutation has important implications as a molecular tool for considering immune therapy responsiveness in ccRCC and possibly across other cancer types.[142]

The Role of HRD as an Emerging Biomarker

Repair of DNA double-strand breaks by cells is mediated by the HR pathway or nonhomologous end-joining. HR is a complex DNA repair pathway involving multiple steps and has been reviewed extensively.[143,144] The *BRCA1* and *BRCA2* genes are critical for efficient double-strand DNA repair via HR and play an important role in the development and clinical progression of many cancers.[145,146]

If a cell carries *BRCA1/BRCA2* LOF mutations, it loses the ability to repair double-strand breaks by HR and is termed the HRD pathway. Such HRD cells are highly sensitive to DNA-damaging agents, such as platinum-based chemotherapies and other cytotoxic agents that can cause DNA strand breaks.[147,148] PARP plays a major role in DNA strand break repair. If PARP is inhibited, then cells ultimately die. Thus combining cytotoxic therapy with a PARP inhibitor can cause cell lethality.

Apart from mutations in the *BRCA1* and *BRCA2* genes, there are several other mechanisms associated with HRD. Defects in HR repair can be because of epigenetic changes such as *BRCA1* promoter methylation, somatic mutations in key HR-related genes, and frequent copy number alterations.[149] In addition, mutations in other genes may result in HR-defective tumors and include but are not limited to *PALB2, RAD51, CHEK2,* and *ATM*.[150-153]

The most common approach to test for HRD is genomic testing for alterations in *BRCA1* and *BRCA2* on the basis that *BRCA1* and *BRCA2* germline and somatic mutations are known to cause HRD. Testing for additional genes involved in DNA damage repair through HR can also be done through commercial resources. Several other approaches have been developed to measure tumor DNA repair function.[154] The myChoice HRD test is an NGS assay that uses DNA extracted from formalin-fixed, paraffin-embedded or frozen tumor tissue. A tumor can be characterized as HR-deficient or HR-nondeficient by combining the HRD score that it generates and its *BRCA1/BRCA2* mutation status. HRD is defined as an HRD score ≥42 or the presence of a mutation in *BRCA1/BRCA2*. As an example of its accuracy, the myChoice HRD assay was seen to identify 100% of BRCA-mutated tumors and 57% of non–BRCA-mutated tumors that had HR deficiencies in patients with platinum-sensitive, high-grade, serous or BRCA-mutated, recurrent ovarian cancer.[65]

The FoundationFocus CDx *BRCA* (Foundation Medicine, Inc) assay was used to detect both germline and somatic *BRCA1/BRCA2* mutation types associated with response to PARP inhibitor therapy.[155,156] This modified NGS-based assay determined the percentage of genomic loss of heterozygosity, mutations in *BRCA1/BRCA2*, and other HR genes in tumor tissue of patients with ovarian cancers taking part in the ARIEL PARP inhibitor rucaparib trial. A prespecified cutoff of ≥14% for high loss of heterozygosity was determined. FoundationFocus CDx *BRCA* is the first FDA-approved companion diagnostic assay for rucaparib for the treatment of advanced ovarian cancer.

As we understand more about HRD in various cancer types, the indications for the use of PARP inhibitors will likely be broadened. Certain cancers, including ovarian, fallopian tube, breast, primary peritoneal, and GI (specifically a subgroup of pancreatic adenocarcinomas and gastric/esophageal cancers), have been shown to harbor aberrations in genes involved in the HRD pathway. Mutations are seen not only in *BRCA1* and *BRCA2* but also in other relevant genes, such as *RAD51, RAD54, DSS1, RPA1, NBN, ATR, ATM, CHK1, CHK2, FANCD2, FANCA*, or *FANCC*.[157,158] Several PARP inhibitors have been FDA approved for the treatment of specific types of ovarian (olaparib, rucaparib, and niraparib), fallopian tube (olaparib and niraparib), breast (olaparib), primary peritoneal (olaparib and niraparib), and pancreatic (olaparib) cancers, but not yet for other GI cancers. However, at present, HRD testing before PARP-inhibitor therapy is not necessary.

Other Hot Markers in Research

Although it is not by any means an exhaustive list, some exciting new biomarkers and their targeted therapies are discussed below.

NTRK and Entrectinib

Entrectinib (RXDX-101) was granted a breakthrough therapy designation by the FDA in 2017 for use as a treatment for adult and pediatric patients with *NTRK*-positive, locally advanced or metastatic solid tumors who have either progressed after prior therapies or who have no acceptable standard therapy options (see also Neurotrophic receptor tyrosine kinase, above).[27,28] A trial studying the treatment of patients with solid tumors (breast cancer, cholangiocarcinoma, CRC, head and neck neoplasms, melanoma, neuroendocrine tumors, NSCLC, ovarian cancer, pancreatic cancer, papillary thyroid cancer, primary brain tumors, renal cell carcinoma, and sarcomas) that harbor an *NTRK1/NTRK2/NTRK3*, *ROS1*, or *ALK* fusion is ongoing (ClinicalTrials.gov identifier NCT02568267). In this trial, patients are assigned to different baskets according to tumor type and gene fusion. The primary outcome of the study will be the objective response rate to entrectinib.[27]

NTRK fusions may act as actionable targets in conjunction with other potentially targetable alterations, such as PD-L1–positive or MSI-H status, meaning that therapeutic combinations (TRK inhibitors plus immune checkpoint inhibitors, for example) are a promising strategy.[159]

FGFR and Erdafitinib

The fibroblast growth factor receptor (FGFR) family comprises part of a tyrosine kinase signaling pathway that plays a role in oncogenesis through gene amplification, activating mutations, or translocation in several tumor types. Erdafitinib is an orally administered FGFR family tyrosine kinase inhibitor. Earlier this year, the FDA granted Breakthrough Therapy Designation for erdafitinib in the

treatment of urothelial cancer, which is based on data from a multicenter phase 2 clinical trial focused on evaluating the efficacy and safety of erdafitinib in the treatment of adult patients with locally advanced or metastatic urothelial cancer harboring specific *FGFR* mutations.[160] The overall response rate was 42% in 59 patients for whom data were available.[160] Erdafitinib is also under investigation in the NCI-MATCH trial as a treatment for patients with tumors that have an *FGFR* mutation, fusion, or amplification (Table 7).

Also in the NCI-MATCH trial, 5 of 50 patients with an aberrant FGFR pathway had a partial response to AZD4547 (another FGFR tyrosine kinase inhibitor).[161] Two of these patient's tumors had point mutations in *FGFR2/FGFR3*, and 2 others had *FGFR3* fusions, suggesting that these particular types of mutation have increased sensitivity to the drug, which warrants further study in this patient subtype.

MET Amplification and *MET* Exon 14 and Crizotinib

Aberrant activation of MET receptor tyrosine kinase signaling occurs in various cancer types as result of various *MET* alterations, including amplification and an exon 14 mutation. Crizotinib is an ALK/ROS1/MET inhibitor that is already FDA approved in *ALK*-positive or *ROS1*-positive NSCLC but also has proven clinical activity in cases of *MET* exon 14 alterations and *MET* amplification. Preclinical studies have shown that inhibition of MET using crizotinib resulted in the inhibition of growth of cancer cells that possessed *MET* amplification both in vitro in cell lines and in vivo in preclinical models.[162] In an updated phase 1 analysis of crizotinib in patients with low, medium, and high levels of *MET* amplification in advanced NSCLC, patients with high *MET* amplification showed clinically meaningful antitumor activity with rapid and durable responses. Crizotinib was generally well tolerated[163] and is currently under study in the ASCO TAPUR trial for patients with tumors that have *ALK*, *ROS1*, or *MET* mutations and in the NCI-MATCH trial as a treatment for patients with tumors that have a *MET* amplification, *MET* exon 14 mutation, *ALK* translocation, or *ROS1* translocation or inversion (Table 7).

mTOR and Sapanisertib (TAK-228)

The mammalian target of rapamycin (mTOR) is a kinase encoded in humans by *MTOR*. mTOR exists as a core component in 2 distinct multiple-protein complexes, TORC1 and TORC2. These complexes regulate several different cellular processes, including cell proliferation, cell motility, cell survival, protein synthesis, autophagy, and transcription. Sapanisertib (TAK-228) demonstrated a reasonable safety profile as well as promising preliminary antitumor activity in a range of tumor types with aberrant *MTOR*.[164] Tuberous sclerosis complex 1 and 2 (*TSC1* and *TSC2*) mutations are also observed in certain tumor subtypes and may be targeted by sapanisertib. The agent is under investigation in the NCI-MATCH trial as a treatment for patients with tumors that have *MTOR* or *TSC1/TSC2* mutations (Table 7).

PIK3CA and Taselisib

In the NCI-MATCH trial, 65 patients with a mutated phosphatidylinositol 3-kinase gene (*PIK3CA*) were treated with taselisib (a PIK3CA inhibitor) and, although there were no ORs to the drug, 24% of patients had prolonged stable disease for more than 6 months. Further research in selected cancer types is warranted.[165]

CDK4/CDK6 and Palbociclib

The cyclin-dependent kinases (CDKs) CDK4 and CDK6 play a crucial role in the G1-S phase transition during cell cycling. Palbociclib, an inhibitor of aberrant CDK4/CDK6, is FDA approved for the treatment of hormone receptor–positive, HER2-negative, advanced or metastatic breast cancer in combination with an aromatase inhibitor as initial endocrine-based therapy in postmenopausal women.[166] Its effect on certain GI tumors is under investigation in the clinic.[167] Palbociclib is also under investigation in the NCI-MATCH trial as a treatment for patients with tumors that have *CDK4* or *CDK6* amplification or *CCND1*, *CCND2*, or *CCND3* amplification (and Rb expression/protein in both study arms). The ASCO TAPUR trial is also investigating palbociclib in the treatment of patients with tumors that harbor *CDKN2A*, *CDK4*, or *CDK6* amplifications (Table 7).

DDR2 and Dasatinib

DDR2 is a transmembrane receptor tyrosine kinase that plays a role in cancer progression by regulating the interactions of tumor cells with their surrounding collagen matrix. *DDR2* mutations are seen in several tumor types, including lung cancer, breast cancer, brain cancer, gynecologic cancer, and prostate cancer.[168] The multikinase inhibitor dasatinib blocks DDR2 kinase activity to various degrees and is under investigation in the treatment of patients with tumors that possess a *DDR2* S768R, I638F, or L239R mutation (NCI-MATCH). The agent is also under investigation in the treatment of patients with tumors that harbor *Bcr-abl*, *SRC, KIT, PDGFRB, EPHA2, FYN, LCK,* or *YES1* mutations (TAPUR trial) (Table 7).

Emerging Techniques

The Liquid Biopsy: Circulating Tumor Cells and Exosomes

Peripheral blood samples are a biomarker source by way of circulating tumor cells (DNA) and circulating nucleic acids or associated extracellular vesicles or exosomes.[169-171] The use of liquid biopsy profiling has proven useful in selected clinical scenarios but, to date, despite its potential in the

management of patients with most metastatic solid tumors, this technique has not established a firm role in standard practice. Obvious advantages include ease of access to the tissue through a simple blood draw. An additional advantage is that circulating samples may help reduce the problem of tumor heterogeneity as it reflects the sum total of the tumor. However, when blood and tumor tissue are concurrently collected and analyzed, results from circulating tumor cell analyses do not always match those obtained from tumor tissue analyses and thus are not always reliable.

Regarding extracellular vesicles or exosomes, a newly developed, minimally invasive ADAPT Biotargeting System characterizes complex biological systems in their inherent state(s) and relies on the fact that a large number of cells in the body secrete extracellular vesicles into the circulation, and the molecular composition of these "exosomes" correlates with the cell of origin. Through intercellular communication, exosomes play a part in controlling many tumor progressive processes, including immune evasion, angiogenesis, and metastasis.[171] The ADAPT assay has been shown to have potential for biomarker identification and therapeutic use across most cancer types.[171,172]

Community Hospital Molecular Testing and Assessment Program

Because 85% of cancer care is delivered in a community setting, it is imperative that the programmatic decisions concerning molecular testing for cancer include standardization, physician engagement, and application to point of care. Hoag Hospital (a large community hospital in Newport Beach, California) decided to embark on an initiative within the context of precision oncology and identified the need for molecular testing. A committee of interested physicians (including molecular pathologists) and administrators was formed, and a request for proposal was developed and put to several CLIA-certified vendors to outsource genomic testing. Certain specific qualifications were emphasized, such as price point; turnaround time; results reporting and support structure; portfolio of testing, including NGS, CNV, and IHC for protein analysis and fusion genes (all preferably using disease-specific panels); and preauthorization and billing services. Several meetings were required to reach a final decision. Once the vendor was selected and contracting was optimized, all tumor molecular testing was standardized, and all ordering and tissue processing was sent through the central pathology laboratory. It is important to note that this process had immediate benefit for the cancer programs because, before this arrangement, molecular testing was haphazard. Tumor tissue samples were being sent by individual physicians to multiple different laboratories/vendors without standardization of tissue collection and processing, and subsequent result reports were generally faxed and unavailable when needed for valuable treatment assessment in the standard clinical or research settings.

After vetting and education concerning this new molecular testing and assessment program at disease site committee and tumor board meetings, it was further decided that genomic testing would be reflexed and ordered by pathology for selected clinical stages of solid tumors. The initial pilot project for this reflex testing was in NSCLC stages IB through IV. Before this initial pilot of genomic reflex testing in lung cancer, approximately 50% of the tumors in patients with advanced lung cancer were being tested for even the minimal NCCN guideline biomarkers. Now, over 95% of advanced lung cancers undergo genomic profiling evaluation that has resulted in pervasive use at the point of care. An illustrated example of this was a recent patient's lung cancer demonstrating an actionable mutation in *BRAF*. The success of this pilot study in reflex profiling has expanded to other cancer disease sites, such as advanced head and neck cancers, ovarian cancers, glioblastoma multiforme, sarcoma, and rare tumors. Over the past 18 months, more than 300 tumors have undergone clinical grade genomic profiling, and those data are readily available to the treating physician through web access. Importantly, physician education is a key component to the establishment of a comprehensive cancer molecular genomics program. Initiation of the more routine use of molecular markers and genomic profiling has stimulated interest and participation in the expanding clinical applications. Along with the assistance of molecular pathology, presentation of genomic data is now frequently requested, and this provides points of discussion in the cancer disease site tumor board meetings. In addition, this information has led to optimal patient selection and a definitive increase in referrals to the phase 1 clinical trials program.

Despite the successful implementation of this intuitional program in cancer molecular testing, challenges remain. These fall into several categories, such as reimbursement issues, evaluating the tumor genomic data for potential germline testing, paring results to clinical trial opportunities, and collecting and collating the genomic data to clinical information and outcomes, as well as the incorporation of new opportunities such as sequencing cell-free DNA or routine pharmacogenetic testing. The overall goal of this program was to provide added value and physician engagement in precision oncology. This is now a work in progress, but we are already evaluating the growth opportunity to enhanced patient care.

Challenges and Open Questions in Molecular Profiling

Absence of Randomized, Controlled Clinical Trials

One of the major challenges with the use of large panels or whole genome sequencing is the absence of randomized

clinical trials demonstrating benefit. Although some retrospective analyses have appeared promising, in general, the field demands more evidence given the expense of drugs and genomic tests and potential harm from exposing patients to toxicity of drugs without proof of efficacy. A properly performed randomized clinical trial requires a large number of patients and needs to be a national study requiring significant resources to cover costs of testing, cost of drugs, and data analysis. It would be problematic to randomize patients to genomic profiling versus no testing. It is also complicated to compare standard therapy with molecularly assigned therapy in terms of comparing apples to apples and defining what the valid endpoints should be. It is clear that the gold standard is OS, but this may be affected by multiple lines of therapy and ultimate use of targeted therapy beyond a particular study. PFS is a reasonable endpoint but, if the randomization occurs at a time when there is effective standard therapy, then the impact of molecularly targeted therapy may be underestimated. With the era of precision oncology, there is opportunity to break new ground in trial design. In this regard, pooling N-of-one data that account for other factors described below, such as tumor heterogeneity, may allow for useful evidence to help patients even if it does not rise to randomization. In addition, the PFS ratio as defined by Von Hoff remains a good metric to determine benefit of molecularly assigned therapy.

Lack of Drugs

Another major challenge is the lack of availability of drugs for numerous drivers of various cancers. Examples of major drivers for which there are currently no approved drugs include mutated β-catenin, mutated *P53*, or mutated *RAS*, among others. On the optimistic side, as drugs are discovered and developed, they can be offered retrospectively to patients who have actionable mutations. A related issue is the lack of drugs that effectively target emergent resistance mechanisms. There are some exceptions with mutated *EGFR*, *ALK*, or *BCR-ABL*.

Tumor Heterogeneity

It is clear that advanced cancers, especially those that have been treated, harbor significant tumor heterogeneity. This includes intralesion heterogeneity, interlesion heterogeneity, and interpatient heterogeneity, all of which complicate treatment recommendations and outcomes of studies.

Platform Heterogeneity

In clinical practice, there are several different available platforms for molecular profiling; each test has its own sensitivity and specificity. The scope of the testing varies in the number of genes, whether RNA or protein expression is assessed, and whether actionable fusions are readily detectable. Such heterogeneity makes it difficult to pool data from different platforms. The various commercial platforms or those performed within academic institutions are continuously evolving, further complicating the issue of platform heterogeneity. Thus older platforms that did not capture actionable targets for which there are effective therapies lead to data sets that may underestimate the value of genomic testing with respect to patient outcomes. Moreover, no study to date has shown that larger panels are worth doing over smaller targeted gene panels that are part of standard of care (eg, *KRAS*, *NRAS*, *BRAF*, and MSI in CRC). However, it is not unreasonable to expect that the many genomic changes representing the tail end of the curve of drivers may affect patient outcomes, especially if there are available drugs that target their pathways. With the emergence and popularity of liquid biopsy, this yet further adds complexity to the platforms. Of note, in the TAPUR study, which models real-life situations, liquid biopsy is acceptable for the identification of actionable targets to allow enrollment in a treatment arm.

CLIA-approved laboratories offering molecular panel analysis

Before a patient sample of any kind can be tested, the assay in question must be validated in a CLIA-certified laboratory. CLIA defines a clinical laboratory as any facility that performs laboratory testing on specimens derived from humans for the purpose of providing information for the diagnosis, prevention, or treatment of a disease or impairment. The CMS regulates all laboratory testing performed on humans in the United States through the CLIA program. In total, CLIA covers approximately 260,000 laboratory entities. The Division of Clinical Laboratory Improvement and Quality, within the Quality, Safety, and Oversight Group under the Center for Clinical Standards and Quality, has responsibility for implementing the CLIA program. The objective of the CLIA program is to ensure quality laboratory testing. Although all clinical laboratories must be properly certified to receive Medicare or Medicaid payments, CLIA has no direct Medicare or Medicaid program responsibilities.

Clinical laboratories must constantly evolve their test offerings to support the most recent advances in cancer care. For NGS tumor profiling assays, there are multiple commercially available kits with similar claims for gene content and sensitivity. Many factors contribute to the decision of which assays or customized solutions will best meet the needs of the laboratory, clinicians, and patient population. Kit costs, capital equipment expenditures, complexity of workflow, and turnaround time are all important factors that can be relatively easily compared and assessed between

assay systems. However, the more important parameters for determining effective, personalized treatment for patients are accuracy, reproducibility, and sensitivity of the assay, and these can be much more challenging to critically evaluate but must be rigorously validated.[173-176] The use of a highly multiplexed, consistent, and well-characterized reference material greatly facilitates the comparison of assay systems.[177]

Data Sharing

Given the numerous challenges described above and others, including limitations of electronic medical records, issues with intellectual property, commercial interests, Health Insurance Portability and Accountability Act of 1996 (HIPAA) regulations, and quality of clinical outcomes data, there are major difficulties with data sharing to expand data sets through larger sample size. In this regard, the Caris Precision Oncology Alliance has been addressing some of the issues through the CODE database, which is increasing the number of patients for whom analysis of clinical outcomes is possible as a function of clinical, genomic, or drug use. Other initiatives of national prominence include Orion, Project GENIE, the WIN Consortium, the Precision Medicine Exchange Consortium, and the Memorial Sloan Kettering (MSK) Cancer Alliance.

Timing

The ideal time to perform genomic testing to maximize its therapeutic value to individual patients with cancer remains a matter of controversy, and the evidence base on which to make recommendations is still evolving. Initially, physicians mainly ordered testing in patients with advanced disease who had exhausted standard-of-care treatment options to help inform choices for treatment with experimental agents. Trials are now underway in groups of patients with earlier stages of disease. For example, the newly activated, NCI-sponsored intergroup stage III colon cancer adjuvant therapy trial randomizes patients to standard adjuvant therapy with FOLFOX or FOLFOX plus experimental treatment with a PD-1 inhibitor. Only patients with MSI-H, stage III colon cancer will be eligible for randomization, and the eligibility determination mandates genomic testing for defective MMR in patients with localized disease. Commercial genomic testing now includes MSI testing in addition to a battery of genes relevant to tumor progression. The addition of MSI testing to these panels is a consequence of the recent approval of pembrolizumab and nivolumab. The agents are currently approved for the treatment of refractory tumors of any histology that exhibit defective MMR.[141]

In patients with advanced cancers, it is clear that the tumor genome continues to evolve with time and with the pressure exerted on cells by treatments that selectively favor the growth of treatment-resistant subclones.[178] Investigators remain concerned about clonal evolution and often will recommend rebiopsy of tumors when patients have refractory disease to ensure that the genomic analysis used to make informed decisions about clinical trials with targeted agents is reflective of the tumor's current biology.[179] Others suggest testing at the first sign of advanced disease, as the efficacy of conventional chemotherapy is variable and strategies for salvage therapy may be required sooner rather than later. The use of "liquid biopsies" either on circulating tumor cells or on cell-free DNA has been touted as a method of assessing the tumor genome without the need for a repeat, invasive biopsy. This remains a research tool at this time and is not generally a part of clinical practice. Continued data analyses are urgently needed and will inevitably occur as more samples are tested and the practical application of these assessments are translated into treatment decisions.

Discussion

In 2019, optimum cancer care requires state-of-the-art molecular diagnostics, a solid knowledge base to interpret and apply the results, and a nearly constant awareness of changes on the horizon. The field is moving that quickly. Comprehensive testing performed on our patients at the beginning of 2019 is likely to be incomplete today. Drug approvals are no longer based solely on large phase 3 trials; these late-stage trials are being replaced by "basket" and "umbrella" trials, allowing us to ensure that the right drug is given to the right patient faster. Subsequent new regulatory and payer approvals seem to come daily. Precision medicine is now a part of our standard practice, but with this comes many new challenges. How do we deal with tumor heterogeneity, and will liquid biopsies satisfactorily replace tissue-based testing? Are we justified in, and can we afford, testing a large sample of patients, knowing that we will only rarely find the sought after "needle in the haystack?" How do we manage our patients' expectations when there is so much press and hype surrounding our new discoveries? Can we afford to develop and ultimately to pay for increasingly expensive therapies targeting increasingly smaller proportions of patients? There is, of course, no turning back, but there is much work ahead. ■

Acknowledgements: Marion L. Hartley, PhD, provided invaluable writing and editing support, table and bibliography creation, author coordination, and article organization. These medical writing activities were supported by Caris Life Sciences.

References

1. Centers for Medicare & Medicaid Services. CMS finalizes coverage of next generation sequencing tests, ensuring enhanced access for cancer patients [press release]. Baltimore, MD: Centers for Medicare & Medicaid Services; 2018. cms.gov/newsroom/press-releases/cms-finalizes-coverage-next-generation-sequencing-tests-ensuring-enhanced-access-cancer-patients. Accessed February 6, 2019.
2. US Food and Drug Administration. FoundationOne CDx-P170019. Silver Spring, MD: US Food and Drug Administration; 2017. fda.gov/medicaldevices/productsandmedicalprocedures/deviceapprovalsandclearances/recentlyapproveddevices/ucm590331.htm. Accessed February 6, 2019.
3. Bhuvaneshwar K, Belouali A, Singh V, et al. G-DOC Plus—an integrative bioinformatics platform for precision medicine. *BMC Bioinformatics.* 2016;17:193.
4. Ciardiello F, Adams R, Tabernero J, et al. Awareness, understanding, and adoption of precision medicine to deliver personalized treatment for patients with cancer: a multinational survey comparison of physicians and patients. *Oncologist.* 2016;21:292-300.
5. Verma M. Personalized medicine and cancer. *J Pers Med.* 2012;2:1-14.
6. Juhl H. Preanalytical aspects: a neglected issue. *Scand J Clin Lab Invest Suppl.* 2010;242:63-65.
7. Lange N, Unger F, Schoppler M, Pursche K, Juhl H, David KA. Identification and validation of a potential marker of tissue quality using gene expression analysis of human colorectal tissue. *PLoS One.* 2015;10:e0133987. doi:10.1371/journal.pone.0133987
8. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology.* 2010;138:2073-2087.e3.
9. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. *N Engl J Med.* 2003;348:919-932.
10. Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determined by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. *Cancer Med.* 2018;7:746-756.
11. Bonneville R, Krook MA, Kautto EA, et al. Landscape of microsatellite instability across 39 cancer types. *JCO Precis Oncol.* 2017;1:1-15. doi:10.1200/PO.17.00073
12. Chang L, Chang M, Kautto HM, et al. Microsatellite instability: a predictive biomarker for cancer immunotherapy. *Appl Immunohistochem Mol Morphol.* 2018;26:e15-e21. doi:10.1097/PAI.0000000000000575.
13. Ott PA, Bang YJ, Berton-Rigaud D, et al. Safety and antitumor activity of pembrolizumab in advanced programmed death ligand 1-positive endometrial cancer: results from the KEYNOTE-028 Study. *J Clin Oncol.* 2017;35:2535-2541.
14. US Food and Drug Administration. FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication.. Silver Spring, MD: US Food and Drug Administration; 2017. fda.gov/drugs/informationondrugs/approveddrugs/ucm560040.htm. Accessed February 6, 2019
15. Pai-Scherf L, Blumenthal GM, Li H, et al. FDA approval summary: pembrolizumab for treatment of metastatic non-small cell lung cancer: firstline therapy and beyond. *Oncologist.* 2017;22:1392-1399.
16. Broderick JM. FDA Approves Nivolumab/Ipilimumab for MSI-H/dMMR Colorectal Cancer Wednesday, Jul 11, 2018. onclive.com/web-exclusives/fda-approves-nivolumabipilimumab-for-msihdmmr-colorectal-cancer.
17. Overman MJ, Lonardi S, Wong KYM, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J Clin Oncol.* 2018;36:773-779. doi:10.1200/JCO.2017.76.9901
18. Gatalica Z, Xiu J, Swensen J, Vranic S Molecular characterization of cancers with NTRK gene fusions. *Mod Pathol.* 2019;32:147-153.
19. Planchard D, Smit EF, Groen HJM, et al. Dabrafenib plus trametinib in patients with previously treated BRAF (V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol.* 2017;18:1307-1316.
20. Li BT, Shen R, Buonocore D, et al. Ado-trastuzumab emtansine in patients with HER2-mutant lung cancers: results from a phase II basket trial. *J Clin Oncol.* 2018;36:2532-2537.
21. Paik PK, Drilon A, Fan PD, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. *Cancer Discov.* 2015;5:842-849. doi:10.1158/2159-8290.CD-14-1467
22. Lee SH, Lee JK, Ahn MJ, et al. Vandetanib in pretreated patients with advanced non-small cell lung cancer-harboring RET rearrangement: a phase II clinical trial. *Ann Oncol.* 2017;28:292-297.
23. Drilon A, Rekhtman N, Arcila M, et al. Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: an open-label, single-centre, phase 2, single-arm trial. *Lancet Oncol.* 2016;17:1653-1660.
24. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *J Mol Diagn.* 2018;20:129-159.
25. Fuse MJ, Okada K, Oh-Hara T, Ogura H, Fujita N, Katayama R. Mechanisms of resistance to NTRK inhibitors and therapeutic strategies in NTRK1-rearranged cancers. *Mol Cancer Ther.* 2017;16:2130-2143.
26. US Food and Drug Administration. FDA approves larotrectinib for solid tumors with NTRK gene fusions. Silver Spring, MD: US Food and Drug Administration; 2018. fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm626720.htm. Accessed February 6, 2019.
27. Drilon A, De Braud FG, Siena S, et al. Entrectinib, an oral pan-Trk, ROS1, and ALK inhibitor in TKI-naïve patients with advanced solid tumors harboring gene rearrangements. Presented at: The 2016 AACR Annual Meeting; April 16-20, 2016; New Orleans, LA. Abstract CT007.
28. FDA Grants Entrectinib Breakthrough Designation for NTRK+ Solid Tumors. onclive.com/web-exclusives/fda-grants-entrectinib-breakthrough-designation-for-ntrk-solid-tumors Published: Tuesday, Accessed May 16, 2017
29. Hechtman JF, Benayed R, Hyman DM, et al. Pan-Trk immunohistochemistry is an efficient and reliable screen for the detection of NTRK fusions. *Am J Surg Pathol.* 2017;41:1547-1551.
30. Hung YP, Fletcher CDM, Hornick JL. Evaluation of pan-TRK immunohistochemistry in infantile fibrosarcoma, lipofibromatosis-like neural tumour and histological mimics. *Histopathology.* 2018;73:634-644.
31. Murphy DA, Ely HA, Shoemaker R, et al. Detecting gene rearrangements in patient populations through a 2-step diagnostic test comprised of rapid IHC enrichment followed by sensitive next-generation sequencing. *Appl Immunohistochem Mol Morphol.* 2017;25:513-523.

32. Lartigue J. Blurring the lines between germline and somatic mutations in cancer. *Oncol Live*. 2017;18. onclive.com/publications/oncology-live/2017/vol-18-no-13/blurring-the-lines-between-germline-and-somatic-mutations-in-cancer. Accessed February 6, 2019.
33. Ding L, Getz G, Wheeler DA, et al. Somatic mutations affect key pathways in lung adenocarcinoma. *Nature*. 2008;455:1069-1075.
34. Yurgelun MB, Kulke MH, Fuchs CS, et al. Cancer susceptibility gene mutations in individuals with colorectal cancer. *J Clin Oncol*. 2017;35:1086-1095.
35. Waddell N, Pajic M, Patch AM, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature*. 2015;518:495-501.
36. Schrader KA, Cheng DT, Joseph V, et al. Germline variants in targeted tumor sequencing using matched normal DNA. *JAMA Oncol*. 2016;2:104-111.
37. Sinicrope FA, Sargent DJ. Clinical implications of microsatellite instability in sporadic colon cancers. *Curr Opin Oncol*. 2009;21:369-373.
38. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med*. 2003;349:247-257.
39. Sargent DJ, Marsoni S, Monges G, et al. Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol*. 2010;28:3219-3226.
40. Sinicrope FA, Foster NR, Thibodeau SN, et al. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst*. 2011;103:863-875.
41. Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med*. 2009;11:35-41.
42. Fuchs CS, Doi T, Jang RWJ, et al. KEYNOTE-059 cohort 1: efficacy and safety of pembrolizumab (pembro) monotherapy in patients with previously treated advanced gastric cancer [abstract]. *J Clin Oncol*. 2017;35(15 suppl):4003.
43. Burn J, Gerdes AM, Macrae F, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet*. 2009;378:2081-2087.
44. Liao X, Lochhead P, Nishihara R, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med*. 2012;367:1596-1606.
45. Karnoub AE, Weinberg RA. Ras oncogenes: split personalities. *Nat Rev Mol Cell Biol*. 2008;9:517-531.
46. Di Nicolantonio F, Martini M, Molinari F, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol*. 2008;26:5705-5712.
47. Passiglia F, Bronte G, Bazan V, Galvano A, Vincenzi B, Russo A. Can KRAS and BRAF mutations limit the benefit of liver resection in metastatic colorectal cancer patients? A systematic review and meta-analysis. *Crit Rev Oncol Hematol*. 2018;99:150-157.
48. Richman SD, Seymour MT, Chambers P, et al. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol*. 2009;27:5931-5937.
49. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol*. 2010;28:466-474.
50. Schirripa M, Bergamo F, Cremolini C, et al. BRAF and RAS mutations as prognostic factors in metastatic colorectal cancer patients undergoing liver resection. *Br J Cancer*. 2015;112:1921-1928.
51. Sinicrope FA, Shi Q, Smyrk TC, et al. Molecular markers identify subtypes of stage III colon cancer associated with patient outcomes. *Gastroenterology*. 2015;148:88-99.
52. Van Cutsem E, Kohne CH, Lang I, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol*. 2011;29:2011-2019.
53. Carter J, Tseng LH, Zheng G, et al. Non-p.V600E BRAF mutations are common using a more sensitive and broad detection tool. *Am J Clin Pathol*. 2015;144:620-628.
54. Tran B, Kopetz S, Tie J, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer*. 2011;117:4623-4632.
55. Robertson AG, Kim J, Al-Ahmadie H, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. *Cell*. 2017;171:540-556, e525.
56. Li Q, Damish A, Frazier ZJ, et al. ERCC2 helicase domain mutations confer nucleotide excision repair deficiency and drive cisplatin sensitivity in muscle-invasive bladder cancer. *Clin Cancer Res*. 2019;25:977-988. doi:10.1158/1078-0432.CCR-18-1001
57. Van Allen EM, Mouw KW, Kim P, et al. Somatic ERCC2 mutations correlate with cisplatin sensitivity in muscle-invasive urothelial carcinoma. *Cancer Discov*. 2014;4:1140-1153.
58. Antonarakis ES, Lu C, Luber B, et al. Germline DNA-repair gene mutations and outcomes in men with metastatic castration-resistant prostate cancer receiving first-line abiraterone and enzalutamide. *Eur Urol*. 2018;74:218-225.
59. Lu E, Thomas GV, Chen Y, et al. DNA repair gene alterations and PARP inhibitor response in patients with metastatic castration-resistant prostate cancer. *J Natl Compr Canc Netw*. 2018;16:933-937.
60. De Bono JS, Goh JCH, Ojamaa K, et al. KEYNOTE-199: pembrolizumab (pembro) for docetaxel-refractory metastatic castration-resistant prostate cancer (mCRPC) [abstract]. *J Clin Oncol*. 2018;36(15 suppl):5007.
61. Carroll PR, Parsons JK, Andriole G, et al. NCCN Guidelines Insights: Prostate Cancer Early Detection, Version 2.2016. *J Natl Compr Canc Netw*. 2016;14:509-519.
62. Society of Gynecologic Oncology (SGO). SOG Clinical Practice Statement: Screening for Lynch Syndrome in Endometrial Cancer. Chicago, IL: Society of Gynecologic Oncology; 2014. sgo.org/clinical-practice/guidelines/screening-for-lynch-syndrome-in-endometrial-cancer. Accessed February 6, 2019.
63. Na R, Zheng SL, Han M, et al. Germline mutations in ATM and BRCA1/2 distinguish risk for lethal and indolent prostate cancer and are associated with early age at death. *Eur Urol*. 2017;71:740-747.
64. Slomovitz BM, Jiang Y, Yates MS, et al. Phase II study of everolimus and letrozole in patients with recurrent endometrial carcinoma. *J Clin Oncol*. 2015;33:930-936.
65. Mirza MR, Monk BJ, Herrstedt J, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. *N Engl J Med*. 2016;375:2154-2164.
66. Coleman RL, Oza AM, Lorusso D, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after

response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2017; 390:1949-1961.

67. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med*. 2012;366:1382-1392.
68. Moore K, Colombo N, Scambia G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N Engl J Med*. 2018;27:2495-2505. doi:10.1056/NEJMoa1810858
69. Burger RA, Brady MF, Bookman MA, et al. Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N Engl J Med*. 2011;365:2473-2483.
70. Oza AM, Cook AD, Pfisterer J, et al. Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. *Lancet Oncol*. 2015;16:928-936.
71. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol*. 2014;15:852-861.
72. Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM, Bhargava R. Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. *Mod Pathol*. 2010;23:205-212.
73. Gucalp A, Tolaney S, Isakoff SJ, et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. *Clin Cancer Res*. 2013;19:5505-5512.
74. Schiavon G, Hrebien S, Garcia-Murillas I, et al. Analysis of ESR1 mutation in circulating tumor DNA demonstrates evolution during therapy for metastatic breast cancer. *Sci Transl Med*. 2015;7:313ra182.
75. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol*. 2016;131:803-820.
76. Stupp R, Brada M, van den Bent MJ, Tonn JC, Pentheroudakis G; ESMO Guidelines Working Group. High-grade glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2014;25(suppl_3): iii93-iii101.
77. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO Classification of Tumours of the Central Nervous System. *Acta Neuropathol*. 2007;114:97-109.
78. Donahue B, Scott CB, Nelson JS, et al. Influence of an oligodendroglial component on the survival of patients with anaplastic astrocytomas: a report of Radiation Therapy Oncology Group 83-02. *Int J Radiat Oncol Biol Phys*. 1997; 38:911-914.
79. van den Bent MJ. Interobserver variation of the histopathological diagnosis in clinical trials on glioma: a clinician's perspective. *Acta Neuropathol*. 2010;120:297-304.
80. Ramkissoon SH, Bi WL, Schumacher SE, et al. Clinical implementation of integrated whole-genome copy number and mutation profiling for glioblastoma. *Neuro Oncol*. 2015;17:1344-1355.
81. Tabone T, Abuhusain HJ, Nowak AK, Erber WN, McDonald KL. Multigene profiling to identify alternative treatment options for glioblastoma: a pilot study. *J Clin Pathol*. 2014;67:550-555.
82. Blumenthal DT, Dvir A, Lossos A, et al. Clinical utility and treatment outcome of comprehensive genomic profiling in high grade glioma patients. *J Neurooncol*. 2016;130:211-219.
83. Cook PJ, Thomas R, Kannan R, et al. Somatic chromosomal engineering identifies BCAN-NTRK1 as a potent glioma driver and therapeutic target. *Nat Commun*. 2017;8:15987.
84. Alvarez-Breckenridge C, Miller JJ, Nayyar N, et al. Clinical and radiographic response following targeting of BCAN-NTRK1 fusion in glioneuronal tumor. *NPJ Precis Oncol*. 2017;1:5.
85. Lassaletta A, Zapotocky M, Mistry M, et al. Therapeutic and prognostic implications of BRAF V600E in pediatric low-grade gliomas. *J Clin Oncol*. 2017;35:2934-2941.
86. Dabrafenib effective in pediatric glioma. *Cancer Discov*. 2017;7:OF5.
87. van den Bent M, Gan HK, Lassman AB, et al. Efficacy of depatuxizumab mafodotin (ABT-414) monotherapy in patients with EGFR-amplified, recurrent glioblastoma: results from a multi-center, international study. *Cancer Chemother Pharmacol*. 2017;80:1209-1217.
88. Reardon DA, Lassman AB, van den Bent M, et al. Efficacy and safety results of ABT-414 in combination with radiation and temozolomide in newly diagnosed glioblastoma. *Neuro Oncol*. 2017;19:965-975.
89. Lassman AB, van den Bent MJ, Gan HK, et al. Safety and efficacy of depatuxizumab mafodotin + temozolomide in patients with EGFR-amplified, recurrent glioblastoma: results from an international phase I multicenter trial. *Neuro Oncol*. 2019;21:106-114. doi:10.1093/neuonc/noy091
90. Schindler G, Capper D, Meyer J, et al. Analysis of BRAF V600E mutation in 1,320 nervous system tumors reveals high mutation frequencies in pleomorphic xanthoastrocytoma, ganglioglioma and extra-cerebellar pilocytic astrocytoma. *Acta Neuropathol*. 2011;121: 397-405.
91. US Food and Drug Administration. Nivolumab for SCCHN. Silver Spring, MD: US Food and Drug Administration; 2016. fda.gov/drugs/informationondrugs/approveddrugs/ucm528920.htm. Accessed February 6, 2019.
92. US Food and Drug Administration. Pembrolizumab (KEYTRUDA). Silver Spring, MD: US Food and Drug Administration; 2016. fda.gov/drugs/informationondrugs/approveddrugs/ucm515627.htm. Accessed February 6, 2019.
93. Agarwal V, Subash A, Nayar R-C, Rao V. Is EGFR really a therapeutic target in head and neck cancers?. *J Surg Oncol*. 2019;119:685-686. doi:10.1002/jso.25386
94. Wheeler DL, Dunn EF, Harari PM. Understanding resistance to EGFR inhibitors-impact on future treatment strategies. *Nat Rev Clin Oncol*. 2010;7: 493-507.
95. Blaszczak W, Barczak W, Wegner A, Golusinski W, Suchorska WM. Clinical value of monoclonal antibodies and tyrosine kinase inhibitors in the treatment of head and neck squamous cell carcinoma. *Med Oncol*. 2017;34:60.
96. Jung K, Kang H, Mehra R. Targeting phosphoinositide 3-kinase (PI3K) in head and neck squamous cell carcinoma (HNSCC). *Cancers Head Neck*. 2018;3:3.
97. Kobayashi K, Hisamatsu K, Suzui N, Hara A, Tomita H, Miyazaki T. A review of HPV-related head and neck cancer. *J Clin Med*. 2018;7:E241.
98. Salama AK, Flaherty KT. BRAF in melanoma: current strategies and future directions. *Clin Cancer Res*. 2013;19: 4326-4334.
99. Gibney GT, Messina JL, Fedorenko IV, Sondak VK, Smalley KS. Paradoxical oncogenesis—the long-term effects of BRAF inhibition in melanoma. *Nat Rev Clin Oncol*. 2013;10:390-399.
100. Long GV, Hauschild A, Santinami M, et al. Adjuvant dabrafenib plus trametinib

in stage III BRAF-mutated melanoma. *N Engl J Med*. 2017;377:1813-1823.

101. Long GV, Flaherty KT, Stroyakovskiy D, et al. Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study. *Ann Oncol*. 2017;28:1631-1639.
102. Beadling C, Jacobson-Dunlop E, Hodi FS, et al. KIT gene mutations and copy number in melanoma subtypes. *Clin Cancer Res*. 2008;14:6821-6828.
103. Bastian BC, Esteve-Puig R. Targeting activated KIT signaling for melanoma therapy. *J Clin Oncol*. 2013;31:3288-3290.
104. Guo J, Si L, Kong Y, et al. Phase II, open-label, single-arm trial of imatinib mesylate in patients with metastatic melanoma harboring c-Kit mutation or amplification. *J Clin Oncol*. 2011;29: 2904-2909.
105. Carvajal RD, Antonescu CR, Wolchok JD, et al. KIT as a therapeutic target in metastatic melanoma. *JAMA*. 2011; 305:2327-2334.
106. Hodi FS, Corless CL, Giobbie-Hurder A, et al. Imatinib for melanomas harboring mutationally activated or amplified KIT arising on mucosal, acral, and chronically sun-damaged skin. *J Clin Oncol*. 2013;31:3182-3190.
107. Fedorenko IV, Gibney GT, Smalley KSM. NRAS mutant melanoma: biological behavior and future strategies for therapeutic management. *Oncogene*. 2017; 32:3009-3018.
108. Dummer R, Schadendorf D, Ascierto PA, et al. Binimetinib versus dacarbazine in patients with advanced NRAS-mutant melanoma (NEMO): a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol*. 2017;18:435-445.
109. Gibney GT, Weiner LM, Atkins MB. Predictive biomarkers for checkpoint inhibitor-based immunotherapy. *Lancet Oncol*. 2016;17:e542-e551.
110. Johnson DB, Frampton GM, Rioth MJ, et al. Targeted next generation sequencing identifies markers of response to PD-1 blockade. *Cancer Immunol Res*. 2016;4:959-967.
111. Hodi FS, Chiarion-Sileni V, Gonzalez R, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol*. 2018;19:1480-1492.
112. Gianni L, Dafni U, Gelber RD, et al. Treatment with trastuzumab for 1 year after adjuvant chemotherapy in patients with HER2-positive early breast cancer: a 4-year follow-up of a randomised controlled trial. *Lancet Oncol*. 2011;12:236-244.
113. Baselga J, Bradbury I, Eidtmann H, et al. Lapatinib with trastuzumab for HER2-positive early breast cancer (NeoALTTO): a randomised, open-label, multicentre, phase 3 trial. *Lancet*. 2012;379:633-640.
114. McNeil C. NCI-MATCH launch highlights new trial design in precision-medicine era. *J Natl Cancer Inst*. 2015; 107:djv193.
115. Mangat PK, Halabi S, Bruinooge SS, et al. Rationale and design of the Targeted Agent and Profiling Utilization Registry (TAPUR) Study. *JCO Precis Oncol*. 2018; 2018. doi:10.1200/PO.18.00122
116. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. *Leukemia*. 2006;20:1467-1473.
117. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014;32:3059-3068.
118. Lister TA, Crowther D, Sutcliffe SB, et al. Report of a committee convened to discuss the evaluation and staging of patients with Hodgkin's disease: Cotswolds meeting. *J Clin Oncol*. 1989;7:1630-1636.
119. European Organization for Research and Treatment of Cancer (EORTC). SPECTA (Screening Patients for Efficient Clinical Trial Access). Recent Developments of the EORTC Collaborative Program Towards Precision Medicine. eortc.org/app/uploads/2017/05/SPECTA-flyer-2015.pdf. Accessed February 6, 2019.
120. Lacombe D, Tejpar S, Salgado R, et al. European perspective for effective cancer drug development. *Nat Rev Clin Oncol*. 2014;11:492-498.
121. European Organization for Research and Treatment of Cancer. The European Organization for Research and Treatment of Cancer Screening Patients for Efficient Clinical Trial Access (EORTC-SPECTA) program. eortc.org/specta/. Accessed February 6, 2019.
122. Yao S, Zhu Y, Chen L. Advances in targeting cell surface signalling molecules for immune modulation. *Nat Rev Drug Discov*. 2013;12:130-146.
123. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity*. 2013;39:1-10.
124. Patel SP, Kurzrock R. PD-L1 expression as a predictive biomarker in cancer immunotherapy. *Mol Cancer Ther*. 2015;14:847-856.
125. Mahoney KM, Atkins MB. Prognostic and predictive markers for the new immunotherapies. *Oncology (Williston Park)*. 2014;28(suppl 3):39-48.
126. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372:2018-2028.
127. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med*. 2016;375:1823-1833.
128. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet*. 2016;387:1540-1550.
129. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015;373: 1627-1639.
130. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373: 123-135.
131. Mansfield AS, Murphy SJ, Peikert T, et al. Heterogeneity of programmed cell death ligand 1 expression in multifocal lung cancer. *Clin Cancer Res*. 2016;22:2177-2182.
132. Chen J, Jiang CC, Jin L, Zhang XD. Regulation of PD-L1: a novel role of pro-survival signalling in cancer. *Ann Oncol*. 2016;27:409-416.
133. Hirsch FR, McElhinny A, Stanforth D, et al. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. *J Thorac Oncol*. 2017;12:208-222.
134. Rimm DL, Han G, Taube JM, et al. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. *JAMA Oncol*. 2017;3:1051-1058.
135. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500: 415-421.
136. Champiat S, Ferte C, Lebel-Binay S, Eggermont A, Soria JC. Exomics and immunogenics: bridging mutational load and immune checkpoints efficacy. *Oncoimmunology*. 2014;3:e27817.

137. Zibelman M, Ramamurthy C, Plimack ER. Emerging role of immunotherapy in urothelial carcinoma—advanced disease. *Urol Oncol.* 2016;34:538-547.

138. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med.* 2014;371:2189-2199.

139. Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science.* 2015;350:207-211.

140. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science.* 2015;348:124-128.

141. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med.* 2015;372:2509-2520.

142. Miao D, Margolis CA, Gao W, et al. Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma. *Science.* 2018;359: 801-806. doi:10.1126/science.aan5951

143. Helleday T. Homologous recombination in cancer development, treatment and development of drug resistance. *Carcinogenesis.* 2010;31:955-960.

144. Li X, Heyer WD. Homologous recombination in DNA repair and DNA damage tolerance. *Cell Res.* 2008;18:99-113.

145. Lord CJ, Ashworth A. BRCAness revisited. *Nat Rev Cancer.* 2016;16:110-120.

146. Krejci L, Altmannova V, Spirek M, Zhao X. Homologous recombination and its regulation. *Nucleic Acids Res.* 2012;40: 5795-5818.

147. Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. *Nat Rev Cancer.* 2004;4:814-819.

148. Silver DP, Livingston DM. Mechanisms of BRCA1 tumor suppression. *Cancer Discov.* 2012;2:679-684.

149. Watkins JA, Irshad S, Grigoriadis A, Tutt AN. Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. *Breast Cancer Res.* 2014;16:211.

150. Antoniou AC, Foulkes WD, Tischkowitz M. Breast-cancer risk in families with mutations in PALB2. *N Engl J Med.* 2014;371:1651-1652.

151. Antoniou AC, Casadei S, Heikkinen T, et al. Breast-cancer risk in families with mutations in PALB2. *N Engl J Med.* 2014;371:497-506.

152. Song H, Dicks E, Ramus SJ, et al. Contribution of germline mutations in the RAD51B, RAD51C, and RAD51D genes to ovarian cancer in the population. *J Clin Oncol.* 2015;33:2901-2907.

153. Ramus SJ, Song H, Dicks E, et al. Germline mutations in the BRIP1, BARD1, PALB2, and NBN genes in women with ovarian cancer. *J Natl Cancer Inst.* 2015;107:djv214.

154. den Brok WD, Schrader KA, Sun S, et al. Homologous recombination deficiency in breast cancer: a clinical review. *JCO Precis Oncol.* 2016;2017. doi:10.1200/ PO.16.00031

155. Moschetta M, George A, Kaye SB, Banerjee S. BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer. *Ann Oncol.* 2016;27: 1449-1455.

156. Hennessy BT, Timms KM, Carey MS, et al. Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer. *J Clin Oncol.* 2010;28:3570-3576.

157. McCabe N, Turner NC, Lord CJ, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Res.* 2006;66: 8109-8115.

158. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature.* 2005;434:917-921.

159. Gatalica Z, Xiu J, Swensen J, Vranic S. Molecular characterization of cancers with NTRK gene fusions. *Mod Pathol.* 2019;32:147-153.

160. Loriot Y, Necchi A, Park SE, et al. Erdafitinib (ERDA; JNJ-42756493), a pan-fibroblast growth factor receptor (FGFR) inhibitor, in patients with metastatic or unresectable urothelial carcinoma (mUC) and FGFR alterations: phase 2 continuous versus intermittent dosing [abstract]. *J Clin Oncol.* 2018;36(6 suppl):411.

161. Chae YK, Vaklavas C, Cheng HH, et al. Molecular Analysis for Therapy Choice (MATCH) arm W: phase II study of AZD4547 in patients with tumors with aberrations in the FGFR pathway [abstract]. *J Clin Oncol.* 2018;36(15 suppl):2503.

162. Kawakami H, Okamoto I, Okamoto W, Tanizaki J, Nakagawa K, Nishio K. Targeting MET amplification as a new oncogenic driver. *Cancers (Basel).* 2014;6:1540-1552.

163. Camidge DR, Otterson GA, Clark JW, et al. Crizotinib in patients (pts) with MET-amplified non-small cell lung cancer (NSCLC): updated safety and efficacy findings from a phase 1 trial [abstract]. *J Clin Oncol.* 2018;36(15 suppl):9062.

164. Burris HA 3rd, Kurkjian CD, Hart L, et al. AK-228 (formerly MLN0128), an investigational dual TORC1/2 inhibitor plus paclitaxel, with/without trastuzumab, in patients with advanced solid malignancies. *Cancer Chemother Pharmacol.* 2017;80:261-273.

165. Krop IE, Jegede O, Grilley-Olson E, et al. Results from molecular analysis for therapy choice (MATCH) arm I: taselisib for PIK3CA-mutated tumors [abstract]. *J Clin Oncol.* 2018;36(15 suppl):101.

166. US Food and Drug Administration. Palbociclib (IBRANCE). Silver Spring, MD: US Food and Drug Administration; 2017. fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm549978.htm. Accessed February 6, 2019.

167. Al Baghdadi T, Halabi S, Garrett-Mayer E, et al. Palbociclib (P) in patients (Pts) with pancreatic cancer (PC) and gallbladder or bile duct cancer (GBC) with CDKN2A alterations: results from the Targeted Agent and Profiling Utilization Registry (TAPUR) study [abstract]. *J Clin Oncol.* 2018;36(15 suppl):2532.

168. Xu C, Buczkowski KA, Zhang Y, et al. NSCLC driven by DDR2 mutation is sensitive to dasatinib and JQ1 combination therapy. *Mol Cancer Ther.* 2015; 14:2382-2389.

169. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med.* 2014;6:224ra224.

170. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol.* 2014;32:579-586.

171. Domenyuk V, Zhong Z, Stark A, et al. Plasma exosome profiling of cancer patients by a next generation systems biology approach. *Sci Rep.* 2017;7:42717. doi:10.1038/srep42741

172. Domenyuk V, Gatalica Z, Santhanam R, et al. Poly-ligand profiling differentiates trastuzumab-treated breast cancer patients according to their outcomes. *Nat Commun.* 2018;9:1219.

173. Aziz N, Zhao Q, Bry L, et al. College of American Pathologists' laboratory standards for next-generation sequencing clinical tests. *Arch Pathol Lab Med.* 2015;139:481-493.

174. Gargis AS, Kalman L, Berry MW, et al. Assuring the quality of next-generation sequencing in clinical laboratory practice. *Nat Biotechnol.* 2012;30:1033-1036.

175. Jennings LJ, Arcila ME, Corless C, et al. Guidelines for validation of next-

generation-sequencing-based oncology panels. *J Mol Diagn*. 2017;19:341-365.

176. Schrijver I, Aziz N, Farkas DH, et al. Opportunities and challenges associated with clinical diagnostic genome sequencing: a report of the Association for Molecular Pathology. *J Mol Diagn*. 2012;14:525-540.

177. Sims DJ, Harrington RD, Polley EC, et al. Plasmid-based materials as multiplex quality controls and calibrators for clinical next-generation sequencing assays. *J Mol Diagn*. 2016;18:336-349.

178. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012; 366:883-892.

179. Thierry AR, Pastor B, Jiang ZQ, et al. Circulating DNA demonstrates convergent evolution and common resistance mechanisms during treatment of colorectal cancer. *Clin Cancer Res*. 2017;23: 4578-4591.