Pan-Cancer Molecular Biomarkers

A Paradigm Shift in Diagnostic Pathology

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KEYWORDS

• Molecular pathology • Next-generation sequencing • Targeted therapy • Cancer biomarkers

Key points

- Clinical-grade next-generation sequencing has enabled the accurate identification of pan-cancer biomarkers.
- Targetable molecular alterations are shared between cancers from multiple anatomic sites.
- Some molecular biomarkers predict response to therapy regardless of the histologic diagnosis.
- New approaches to clinical trial design and the regulatory environment contribute to the increasing availability of new molecularly targeted therapies.

SYNOPSIS

The rapid adoption of next-generation sequencing in clinical oncology has enabled the detection of molecular biomarkers shared between multiple tumor types. These pan-cancer biomarkers include sequence-altering mutations, copy number changes, gene rearrangements, and mutational signatures and have been demonstrated to predict response to targeted therapy. This article reviews issues surrounding current and emerging pan-cancer molecular biomarkers in clinical oncology: technological advances that enable the broad detection of cancer mutations across hundreds of genes, the spectrum of driver and passenger mutations derived from human cancer genomes, and implications for patient care now and in the near future.

OVERVIEW

Cancer is traditionally classified based on tissue of origin and the morphologic relatedness between neoplastic cells and normal anatomic structures. This system of classification is embedded within the vocabulary of surgical pathology. The broad differential diagnosis of malignant neoplasms, carcinoma, sarcoma, lymphoma, and so forth, reflects derivation from epithelial, mesenchymal, or hematopoietic cell lineages and forms the basis of all subsequent diagnostic analysis. Traditional pathologic classification is extremely powerful. The anatomic pathologic diagnosis represents the clinical gold standard for informing prognosis and treatment selection.[1]

Advances in molecular diagnostics have identified key genetic alterations that drive the biology of neoplastic disorders. Discoveries of *BCR-ABL1* translocations in chronic myelogenous leukemia and *EGFR* mutations in lung adenocarcinoma have led to the development of small-molecule inhibitors to specifically target the vulnerabilities of tumor cells at a molecular level, ushering in a new era of precision medicine.[2,3] For surgical pathologists, much of the interest in molecular diagnostics has been focused on identifying disease-specific mutations that can assist with traditional classification. However, cancer genetics is complex, and oncogenic mutations are frequently nonspecific and shared across different cancer types. For

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example, recurrent gene rearrangements involving *ALK* are present in anaplastic large-cell lymphoma, inflammatory myofibroblastic tumor, lung adenocarcinoma, and other cancers.[4–6] Targeting *ALK*-rearranged cancers with small-molecule inhibitors has been associated with therapeutic response in multiple cancer types.[7,8]

Several recent approvals of cancer therapies by the Food and Drug Administration (FDA) have been based on molecular biomarkers without consideration of primary tumor site. These approvals represent a paradigm shift in diagnostic pathology and medical oncology. Larotrectinib and entrectinib, tyrosine kinase inhibitors, have been approved for solid tumors with NTRK gene fusions.[9–11] Pembrolizumab, an immune checkpoint inhibitor, has been approved for solid tumors with microsatellite instability[12] as well as solid tumors with high-tumor mutational burden.[13] These therapeutic indications reflect an evolution in clinical practice in which advanced molecular testing, specifically next-generation sequencing, has become standard of care for patients with cancer, and molecular results are more informative than microscopic diagnosis for therapy selection in some situations.

THE EMERGENCE OF PAN-CANCER MOLECULAR BIOMARKERS HAS BEEN ENABLED BY TECHNOLOGICAL ADVANCES IN CLINICAL CANCER SEQUENCING

The concept of pan-cancer molecular biomarkers requires clinical-grade molecular testing platforms that are broadly applicable across multiple cancer types. Before the advent of next-generation sequencing, traditional molecular tests would analyze specific genetic hotspots for the presence of clinically actionable mutations. Lung adenocarcinomas would be specifically evaluated for the presence of *EGFR* L858R, whereas melanomas would be specifically tested for *BRAF* V600E mutation.[14,15] According to these testing strategies, pathologic diagnosis guides molecular test selection. Although a small proportion of lung adenocarcinomas harbor *BRAF* mutations, *BRAF* would not be evaluated in this context because of a lack clinical actionability at that time.

Pan-cancer analysis has been made possible by the widespread adoption of panel next-generation sequencing (**Fig. 1**). Instead of using separate targeted assays for each cancer type, molecular laboratories can now validate a single assay that tests all clinically relevant genes across multiple cancer types. Next-generation sequencing assays applied to targeted cancer genomes detect a variety of somatic mutations, including nucleotide substitutions, insertions and deletions, copy number alterations, and in some situations, structural variants, such as inversions and translocations.[16,17] Multiple laboratory-developed cancer panels have been validated for clinical testing,[18–21] and sequencing library preparation methods have been adapted to detect gene fusion events detectable from messenger RNA.[22] These technological innovations have allowed molecular laboratories to detect a broader range of mutations[23] and consolidate the testing of multiple cancer types

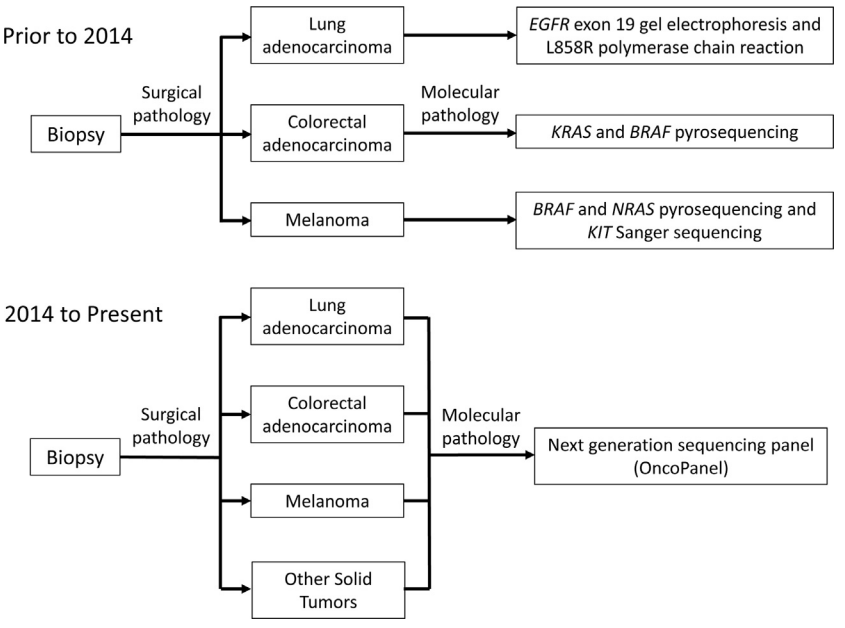

Fig. 1. Clinical molecular test selection at Brigham and Women's Hospital before (*top*) and after (*bottom*) next-generation sequencing.[21] Panel sequencing allows the molecular laboratory to streamline workflow, detect a broader spectrum of mutations, and rapidly initiate clinical testing as new indications for targeted therapies become available.

onto a single platform.[24,25] A College of American Pathologists survey in December 2014 showed that 72% of molecular laboratories had adopted or were planning to adopt next-generation sequencing within the following 2 years.[26] A nationally representative survey of medical oncologists showed that as of 2017, 76% of oncologists in the United States were using next-generation sequencing tests to guide treatment decisions for patients with advanced disease, determine eligibility for clinical trials, and prescribe off-label therapy.[27]

MANY TARGETABLE MUTATIONS ARE SHARED ACROSS TUMORS OF VARIOUS PRIMARY SITES

Large patient cohorts, such as The Cancer Genome Atlas and the International Cancer Genome Consortium, have provided insight into the genomic alterations that drive human cancer development.[28] Although understanding the full ramifications of cancer genomes remains complex, a reductionist perspective has argued that a typical cancer is defined by only 2 to 8 driver mutations, which involve a group of 125 cancer-associated genes in a handful of signaling pathways.[29] Frequently mutated pathways promote functions that are advantageous to tumor cells and have effects on cell survival, cell differentiation, and genome maintenance.

Although each cancer type has a unique distribution of mutations, many frequently mutated oncogenes in potentially targetable pathways are shared among multiple cancer types (**Fig. 2**). Based on these observations, the scientific field hypothesized that the efficacy of targeted therapies might be independent of tumor type as long as a targetable molecular alteration was present. An early test of this hypothesis was disappointing: BRAF small-molecule inhibitors vemurafenib and dabrafenib showed efficacy against melanomas with *BRAF* V600E mutation[30,31] but had little to no efficacy when used as monotherapy against colorectal cancers with the same mutation.[32,33] However, subsequent trials of another BRAF inhibitor encorafenib in combination with cetuximab, an anti-EGFR antibody therapy, showed benefit for patients with metastatic colorectal cancer after failing other therapeutic options.[34] Clinical benefit with combination targeted therapy has also been observed for patients with advanced lung cancers with *BRAF* V600E mutation.[35,36]

Similar treatment strategies have also been applied to other single-gene biomarkers, where standard-of-care therapies in 1 cancer type have been demonstrated to also be effective in another cancer with a lower frequency of the genetic alteration. For example, trastuzumab, a targeted antibody therapy against ERBB2 (HER2) that has long been used for *ERBB2*-amplified breast carcinomas, has also demonstrated efficacy against gastroesophageal,[37] colorectal,[38] and uterine serous carcinomas[39] with *ERBB2* amplification. Mutation biomarkers that have been associated with at least 1 FDA-approved targeted therapy and have evidence of clinical significance in more than 1 tumor type are summarized in **Table 1**.

MOLECULAR BIOMARKERS BEYOND SINGLE MUTATIONS: MUTATIONAL SIGNATURES AND TUMOR MUTATIONAL BURDEN

Next-generation sequencing assays can provide more information than targetable mutational hotspots. In panels large enough to provide representative sampling of the cancer genome, patterns of somatic mutations emerge. Computational techniques by nonnegative matrix factorization have deconvoluted data from large cancer cohorts to derive several unique *mutational signatures*.[40] Mutational signatures reflect the underlying mechanisms of mutagenesis, such as spontaneous deamination of methylated cytosines in sporadic cancers associated with age,[41] dimerization of adjacent pyrimidine nucleic acids by ultraviolet radiation,[42] and the formation of DNA adducts by polycyclic aromatic hydrocarbons in tobacco smoke.[43]

The analysis of mutational signatures has clinical implications in some situations. Some mutational processes generate a high number of somatic mutations during the course of cancer development, and these mutations may be counted as *tumor mutational burden*, defined as the total number of somatic mutations divided by the genomic region covered by the sequencing panel. A higher number of somatic mutations may lead to the translation of novel peptides that can be processed into neoantigens, which may be able to elicit a response from the host immune system. Higher tumor mutational burden has been associated with favorable response to immune checkpoint inhibitor therapy for patients with melanoma,[44] non–small cell lung carcinoma,[45] and other cancer types.[46] Pembrolizumab has been recently approved for advanced solid tumors with high tumor mutational burden, defined as greater than 10 mutations per megabase, regardless of histologic diagnosis.[13]

Another mutational signature with direct clinical implications is microsatellite instability. In cancers with microsatellite instability, inactivation of at least

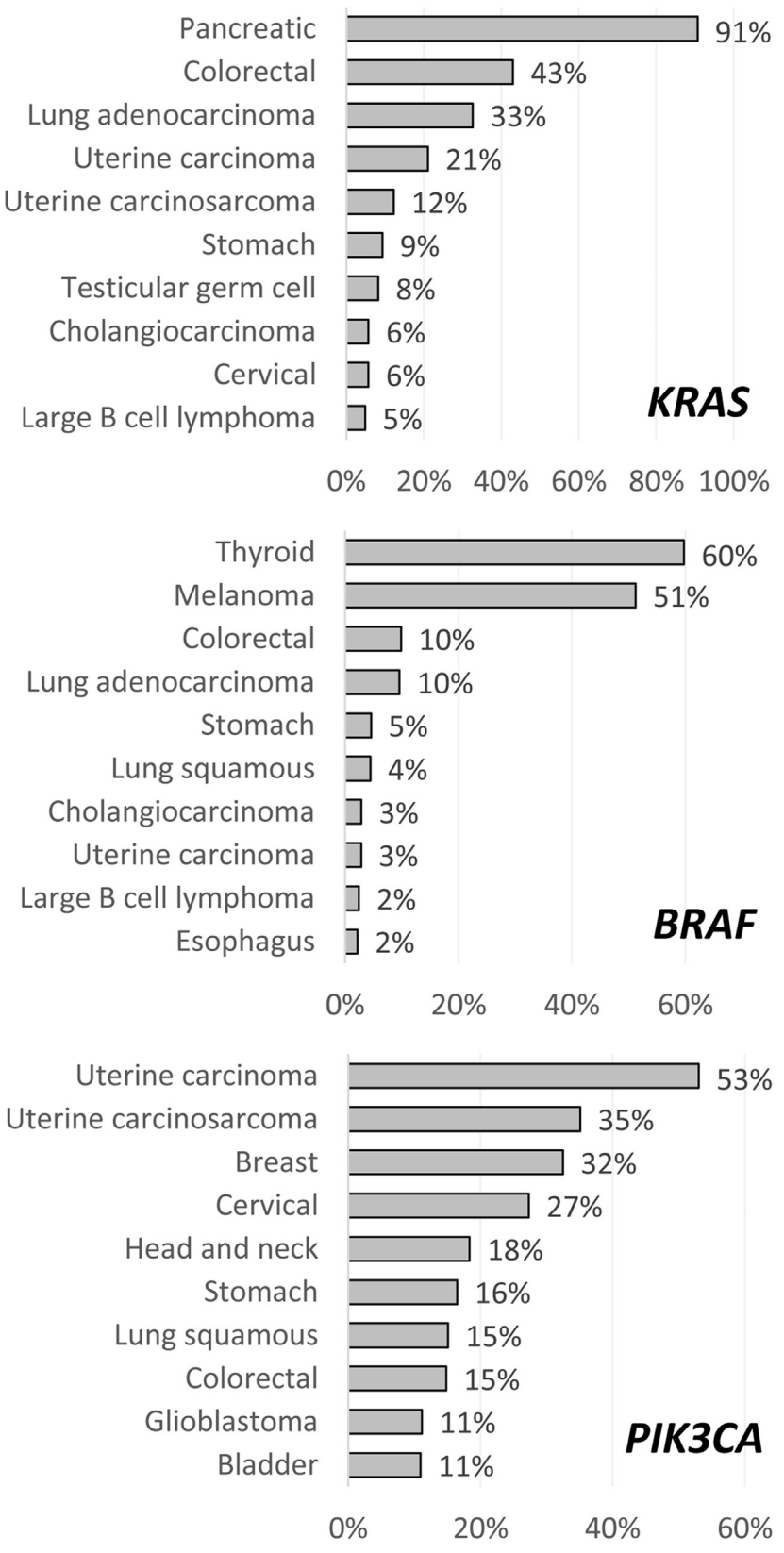

Fig. 2. Frequency of *KRAS*, *BRAF*, and *PIK3CA* mutations across common cancer types. (Data from the Cancer Genome Atlas.[75] From Cerami E, Gao J, Dogrusoz U, et al. The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data: Figure 1. *Cancer Discov.* 2012;2(5):401-404.)

Table 1
Mutational biomarkers with tumor-specific Food and Drug Administration–approved targeted therapies with evidence of clinical actionability in more than 1 cancer type

Gene	Alteration	Cancer Type	Indication	Level of Evidence
BRAF	V600E	Melanoma	Response to vemurafenib or dabrafenib with or without trametinib	FDA approval[30,76,77]
		Lung adenocarcinoma	Response to dabrafenib and trametinib	FDA approval[35,36]
		Colorectal adenocarcinoma	Response to encorafenib and cetuximab	FDA approval[34]
KRAS	Hotspot mutations	Colorectal adenocarcinoma	Lack of response to cetuximab and panitumumab	FDA label[78]
		Lung adenocarcinoma	Mutual exclusivity with other targetable oncogenic gene mutations	Practice guideline[79]
PIK3CA	Hotspot mutations	$ER^+/HER2^-$ breast carcinoma	Response to alpelisib	FDA approval[80]
	H1047R	Mixed	Response to PI3K pathway inhibitors	Clinical trial[81,82]
ERBB2	Amplification	Breast carcinoma	Response to trastuzumab	FDA approval[83,84]
		Gastric adenocarcinoma	Response to trastuzumab	FDA approval[37]
		Colorectal adenocarcinoma	Response to trastuzumab and lapatinib or trastuzumab and pertuzumab	Clinical trial[38,85]
		Uterine serous carcinoma	Response to trastuzumab	Clinical trial[39]
BRCA1 *BRCA2*	Deleterious mutation	Ovarian serous carcinoma	Response to olaparib, niraparib, or rucaparib	FDA approval[86]
		Breast carcinoma	Response to olaparib or talazoparib	FDA approval[87]
		Pancreatic adenocarcinoma	Response to olaparib	FDA approval[88]
		Prostatic adenocarcinoma	Response to rucaparib Response to olaparib	FDA approval Clinical trial[89]
ALK	Gene fusion	Lung adenocarcinoma	Response to crizotinib	FDA approval[8]
		Anaplastic large cell lymphoma	Response to crizotinib	Clinical trial[90]
		Inflammatory myofibroblastic tumor	Response to crizotinib	Clinical trial[7,90]
RET	Gene fusion	Lung adenocarcinoma Thyroid carcinoma	Response to selpercatinib	FDA approval
	Mutation	Medullary thyroid carcinoma		
FGFR2 or FGFR3	Mutation or gene fusion	Bladder cancer	Response to erdafitinib	FDA approval[91]
FGFR2	Gene fusion	Cholangiocarcinoma	Response to pemigatinib	FDA approval[92]

1 of 4 DNA mismatch repair proteins (MLH1, MSH2, MSH6, or PMS2) leads to a characteristic pattern of mutations. A hallmark feature is insertion and deletion mutations in tracts of repetitive DNA (microsatellites). Microsatellite instability was originally described as a shift in the size of the DNA repeat regions detectable by amplification and gel electrophoresis.[47,48] Microsatellite instability can also be inferred from next-generation sequencing data, either by evaluation of incidentally captured microsatellites[49–51] or by evaluation of mutations in short nucleotide repeats in coding regions.[52] These methods have been applied to solid tumors with relatively high rates of microsatellite instability, such as colorectal[53] and endometrial carcinomas,[54] as well as other solid tumors with lower frequencies of microsatellite instability, including upper gastrointestinal tract[55] and prostatic carcinomas.[56] Solid tumors with microsatellite instability have been associated with response to immune checkpoint inhibitor therapy.[57]

An emerging biomarker is a mutational signature in cancers with homologous recombination deficiency. Homologous recombination deficiency is most commonly associated with *BRCA1* or *BRCA2* loss-of-function mutations, but multiple genes play a role in the homologous recombination pathway.[58] Homologous recombination deficiency is associated with improved sensitivity to platinum chemotherapy[59] and is targetable with poly(ADP-ribose) polymerase (PARP) small-molecule inhibitors. A distinctive pattern of somatic mutations has been identified in solid tumors with BRCA1 or BRCA2 deficiency based on the pattern of nucleotide substitutions, deletion mutations, and loss of heterozygosity.[60–63] Genomic findings associated with homologous recombination deficiency have been aggregated in predictive scoring algorithms.[64] These mutational patterns have been postulated to be useful to guide prognosis[65] and predict sensitivity to PARP inhibitors for patients without detectable *BRCA1* or *BRCA2* alterations.[66,67]

NEW CLINICAL TRIAL DESIGN STRATEGIES IN THE VALIDATION OF PAN-CANCER BIOMARKERS

Although advanced molecular diagnostic testing is increasingly important in treatment selection, the clinical validation of new biomarkers does not depend on pathology alone. The availability of targeted therapies developed by pharmaceutical companies plays a major role in the clinical impact of diagnostic testing. Determining the efficacy of new therapies depends on carefully controlled clinical trials and approval by regulatory agencies.

The recent approvals of therapies based on pan-cancer biomarkers are the direct results of shifting trends in clinical trial design. Traditional clinical trials enroll patients based on cancer diagnosis or site of origin. The association between therapeutic response and molecular biomarkers may not be apparent until analysis is performed on molecular subgroups. Limitations of these methods were shown in early trials of EGFR small-molecular inhibitors in lung cancer, which did not demonstrate benefit compared with chemotherapy in a cohort of patients without *EGFR* mutation analysis.[68]

Basket trials directly test the core hypothesis of precision medicine: that therapy selection for patients with cancer should be predicated on molecular biomarkers representing direct targets or targetable phenotypes.[69] Centralized molecular testing, most commonly by next-generation sequencing, stratifies patients into 1 of several arms. Targeted therapy is provided based on molecular phenotype irrespective of the morphologic diagnosis, and patients are followed and evaluated for objective response. Basket trials have an advantage in enrolling patients with uncommon molecular alterations who may be candidates for targeted therapy. A search in PubMed for the term "basket trial" shows a steady increase in publications on this topic since 2015 (**Fig. 3**).

Last, the accelerated availability of targeted therapies associated with molecular biomarkers has been assisted by evolving trends in regulation. In 2012, the US Congress created the "breakthrough therapy" designation to expedite FDA approval for novel therapeutics with preliminary evidence of exceptional benefit. The rationale was to make effective therapies available in a more timely fashion and was in part supported by the success of molecularly targeted therapies.[70] The breakthrough therapy program, along with the accelerated approval track to expedite the availability of therapies that fulfill unmet medical needs, use surrogate endpoints, such as radiographic response, in lieu of primary endpoints, such as disease-specific or overall survival.

Despite the general excitement surrounding expedited drug approval, objective analysis has shown poor correlation between cancer drug approval based on a surrogate end point and subsequent demonstration of improved overall survival in randomized trials.[71,72] The accelerated approval of targeted therapies for tumor-agnostic indications remains controversial,[73,74] and it remains to be seen whether improvements in patient outcome can be demonstrated in randomized trials.

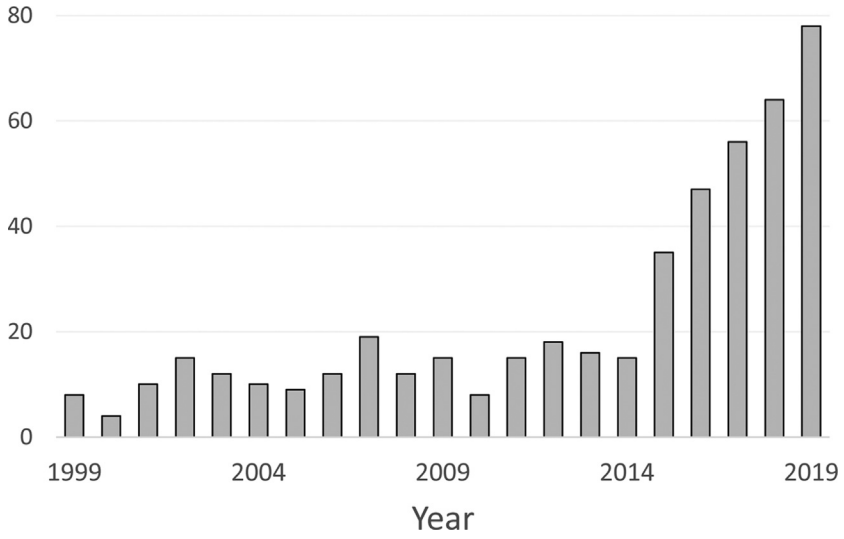

Fig. 3. Number of results on PubMed for the search term "basket trial," showing an increase in related publications since 2015.

SUMMARY

Surgical pathology has long been the standard for informing patient diagnosis and prognosis and directing therapeutic options. The integration of advanced molecular technologies into clinical practice has identified pan-cancer biomarkers that inform response to therapy irrespective of pathologic diagnosis. As of August 2020, the FDA has approved NTRK gene fusions, microsatellite instability, and tumor mutational burden as tumor-agnostic biomarkers linked to response to specific therapies for patients with advanced cancer, and the clinical, academic, pharmaceutical, and regulatory communities are clearly interested in promoting the availability of targeted therapies linked to molecular mechanisms. If current trends continue, there will be a continuing need to integrate results from advanced molecular diagnostic testing with surgical pathology to inform diagnosis and aid treatment decisions for patients with cancer.

CLINICS CARE POINTS

- Molecular diagnostics, including next-generation sequencing, is now routinely incorporated into the care of patients with advanced cancer.
- Advances in molecular diagnostic technology has enabled the clinical utilization of pan-cancer biomarkers.
- Evidence-based interpretation of molecular testing results can aid treatment decisions for patients with cancer.

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