

# Seize the Opportunity

## Underutilization of Fine-Needle Aspiration Biopsy to Inform Targeted Cancer Therapy Decisions

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**Two** years ago, Krishnamurthy presented a comprehensive review on the applications of molecular techniques to fine-needle aspiration (FNA) biopsy samples, noting “the excellent potential of using material procured from FNAB for almost any type of molecular test.”<sup>1</sup> She further observed that ‘whereas few of these tests alone are used for patient care, some of them have the potential for clinical use in the near future’ and “most of the molecular tests using FNAB specimens are currently investigational.” This article will provide an update on the current utilization of FNA-based tests to inform targeted cancer therapy decisions, making the case that FNAs are underutilized for such testing. The emphasis will be on solid tumors rather than sarcomas or lymphoma. Some of the major impediments to the widespread clinical utilization of FNAs for molecular tests will also be identified and possible solutions will be presented.

### ***Molecularly Targeted Agents and Biomarkers***

The recent emergence of targeted therapeutics for cancer offers the hope of personalized cancer care. By definition, “targeted cancer therapies use drugs that block the growth and spread of cancer by interfering with molecular and cellular changes that are specific to cancer.”<sup>2</sup> Although some might consider selective estrogen receptor modulators for breast cancer to be targeted therapy, trastuzumab (Herceptin) is widely regarded as the first true molecularly targeted agent. Since the approval of trastuzumab for advanced breast carcinoma in 1998, 10 additional molecularly targeted agents have been approved for treatment of solid tumors (Table 1). There is growing recognition that these therapies are most effective when given to the subpopulations of patients whose tumors contain the molecular defect targeted by the drug.<sup>3</sup> In general, these molecular defects, or ‘biomarkers,’ are broadly defined as follows: “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.”<sup>4</sup> Biomarker assays that determine whether a person’s cancer will respond to a specific treatment are termed *predictive* tests. These should be distinguished from tests that simply assess an individual’s likelihood of disease progression or death, which are termed *prognostic* tests. To date, predictive tests for solid tumors have focused primarily on the detection of defects in HER2, EGFR, and KRAS (Table 1). These molecular defects may include protein expression (or overexpression)

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**Table 1.** FDA-Approved Molecularly Targeted Therapies for Solid Tumors

Drug	Target	Indication	Biomarker Assay
<b>Small molecule antagonists</b>			
Gefitinib (Iressa)	EGFR	NSCLC	EGFR Gene Mutation; EGFR Protein Expression (IHC)
Erlotinib (Tarceva)	EGFR	NSCLC; Pancreatic Carcinoma	EGFR Gene Mutation; EGFR Protein Expression (IHC)
Sorafenib (Nexavar)	RAF, VEGFR, PDGFR	RCC; HCC	None
Sunitinib (Sutent)	PDGFR, VEGFR, c-KIT, FLT3	RCC, GIST	None
Lapatinib (Tykerb)	EGFR, HER2, ERK1/2, AKT	Breast Carcinoma	HER2 Protein Overexpression (IHC); HER2 Gene Amplification (FISH)
Temsirolimus (Torisel)	mTOR	RCC	None
Everolimus (Afinitor)	mTOR	RCC	None
<b>Monoclonal antibodies</b>			
Trastuzumab (Herceptin)	HER2	Breast Carcinoma	HER2 Protein Overexpression (IHC); HER2 Gene Amplification (FISH)
Bevacizumab (Avastin)	VEGF	CRC; NSCLC; Breast Carcinoma	None
Cetuximab (Erbix)	EGFR	CRC; SCCHN	EGFR Protein Expression (IHC); KRAS Gene Mutation
Panitumumab (Vectibix)	EGFR	CRC	EGFR Protein Expression (IHC); KRAS Gene Mutation

CRC indicates colorectal cancer; EGFR, epidermal growth factor receptor; FLT3, Fms-related tyrosine kinase 3; GIST, gastrointestinal stromal tumor; HCC, hepatocellular carcinoma; mTOR, mammalian target of rapamycin; NSCLC, nonsmall cell lung carcinoma; PDGFR, platelet-derived growth factor receptor; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma head and neck; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Sources: <http://www.cancer.gov/drugdictionary/>; <http://www.fda.gov>

detected by immunohistochemistry (IHC), gene amplification detected by fluorescence in situ hybridization (FISH), or the identification of specific mutations within the genes themselves. There are also emerging tests based on mRNA expression profiles derived from panels of multiple genes.

### Identification of Target Expression and Overexpression

#### HER2

One of the most widely used molecularly targeted agents, trastuzumab, and the newer drug, lapatinib, are indicated for HER2 overexpressing metastatic breast carcinoma. HER2 overexpression is detected in approximately 20% of breast cancers, and approximately 50% of these women have an objective response to trastuzumab.<sup>5-8</sup> Accurate identification of HER2 overexpressing tumors has historically been challenging, and flawed testing has resulted in inappropriate therapy for patients. Flaws in the IHC test have ranged from variable fixation to subjectivity in interpretation.<sup>9</sup> Lessons learned from this experience have highlighted the paradigm shift in the role of IHC from a qualitative test to characterize tumors to a semiquantitative test to guide therapy.<sup>10</sup>

Recent American Society of Clinical Oncology guidelines have stated that samples for HER2 IHC testing should be fixed in 10% neutral buffered formalin for at least 6 hours and no more than 48 hours. Any changes in the test conditions “such as use of alternate fixatives” must be validated against standard methods to demonstrate concordance.<sup>5</sup> Because the standardization of IHC-based HER2 testing was based on formalin-fixed paraffin-embedded (FFPE) core biopsies or surgically excised tissue, cytopathologists have faced hurdles in extending HER2 IHC testing to FNA samples. One of the most commonly utilized FDA-approved kits for HER2 IHC, the HercepTest (Dako), was developed and validated for ‘routinely processed paraffin-embedded specimens that have been fixed in neutral buffered formalin or Bouin’s fixative.’<sup>11</sup> The kit has not been validated for other fixatives. In fact, alcohol-based fixatives, like those commonly used in cytologic specimens, even cell block preparations, may cause false positive results.<sup>12</sup> Other commonly used cytologic preparation techniques such as direct smears or monolayer preparations have not been validated for the HercepTest kit. Although such preparations may yield results, heightened expectations for reproducible and accurate performance characteristics demand extensive and thorough validation.

Other features of the HER2 IHC test that create challenges for cytologic specimens are the interpretation criteria, which are based on histologic sections. Specifically, the HER2 score is based exclusively on the extent of membrane staining, as opposed to cytoplasmic staining. Such distinction may be extremely difficult in cytologic preparations other than cell blocks. The relatively small number of tumor cells in an FNA sample, compared with an excised tumor, may also create scoring problems for HER2 IHC because specimens near a cutoff of 10% positive tumor cells should have a minimum of 100 tumor cells counted, according to the kit manufacturer.

FISH or chromogenic ISH-based HER2 tests are indicated for breast tumors that are HER2 2+ on IHC to determine true HER2 overexpressing tumors through the identification of HER2 gene amplification. It has been suggested that FISH testing be utilized as the primary tool for determining HER2 overexpressing tumors rather than IHC.<sup>13</sup> Like IHC-based tests, these, too, have been developed and validated for FFPE samples rather than noncell block cytologic preparations. The cell numbers required for analysis (20-30 cells) are compatible with typical FNA yields, but because of intra-tumoral heterogeneity in the degree of amplification, sampling artifacts may affect the scoring.

### ***Epidermal Growth Factor Receptor***

In addition to HER2, the epidermal growth factor receptor (EGFR) is a common target for several molecularly targeted agents, including gefitinib, erlotinib, panitumumab and cetuximab. The monoclonal antibodies, panitumumab, and cetuximab, are indicated for EGFR-expressing metastatic colorectal carcinoma (CRC), which represents approximately 75% of cases. Like HER2 overexpressing breast carcinoma, EGFR-expressing CRC can be identified by IHC. The EGFR IHC test also presents similar challenges as HER2 IHC test for cytologic samples. The FDA-approved EGFR IHC test, EGFR PharmDx (Dako), was developed and validated only for FFPE specimens.<sup>14</sup> The interpretive criteria require evaluation of circumferential staining of cell membranes and exclusion of cytoplasmic staining. Such criteria are not readily transferred to traditional cytologic preparations because of the lack of cell sectioning. In addition, membrane staining in  $\geq 1\%$  of tumor cells is considered an

EGFR-expressing tumor, for purposes of cetuximab or panitumumab therapy eligibility. Given the relatively limited sampling by FNA, this raises the possibility of false negative results in limited samples and inappropriate denial of cetuximab or panitumumab therapy.

### ***Gene Mutation Tests***

#### **EGFR**

EGFR mutations are predictive markers for response to EGFR tyrosine kinase inhibitors (gefitinib and erlotinib) in nonsmall cell lung cancer, and they are detected in approximately 10% of lung cancer patients in the United States. Of these, approximately 55%-75% of patients demonstrate a complete or partial response in prospective studies.<sup>15,16</sup> Testing for EGFR mutations typically involves sequencing of polymerase chain reaction (PCR)-amplified EGFR DNA from areas known to harbor mutations. To avoid false negative results, sequencing-based tests may require up to 50%-70% tumor cells in a sample. Such testing may be provided by commercial laboratories, such as Genzyme Genetics, or through individual laboratory developed tests. Genzyme Genetics' Web site states that it will accept "core needle biopsies, FNAs and pleural effusions."<sup>17</sup> They go on to state that "the smaller amount of tissue usually present in these specimens can lower the success rate of the assay." Other more sensitive assays, such as TheraScreen EGFR29 (DxS, Ltd.), are emerging that may provide advantages over sequencing-based tests. The methods used in this kit can purportedly detect <10 copies of mutant DNA in a sample containing just 1% tumor. But such exquisitely sensitive tests raise the concern of detecting rare background mutations that are not relevant to therapeutic choices.

Unfortunately, it appears that relatively few EGFR mutation tests are actually performed on FNA samples. In a recent publication, Smouse et al reviewed 239 samples from the Brigham and Women's Hospital over 2 years and found that only 4 of these samples were from FNAs (plus 7 pleural fluids and 1 BAL).<sup>18</sup> Similarly, we have reviewed our experience at The Johns Hopkins Hospital and found that of 59 specimens tested in 2008, only 6 of these were from FNAs (plus 7 pleural fluids). In contrast, a Swiss study of 84 consecutive cytologic specimens (35 transbronchial FNAs, 15 bronchial washings, 13 bronchial brushes, 5 bronchoalveolar lavages, and 16 pleural

effusions) from nonsmall cell lung carcinoma (NSCLC), which utilized DNA sequencing on laser capture microdissected tumor cells from cytologic preparations, was able to achieve results from 93% of the samples and obtain the best results from samples that contained at least 100 tumor cells.<sup>19</sup> A group from the Memorial Sloan-Kettering Cancer Center has experience in the development and application of EGFR mutation testing of both tissue sections and cytologic clinical samples.<sup>20</sup> In their summary of 334 lung cancer samples from a consecutive 12-month period, they were able to use “some” cell blocks from FNA samples (number not specified) and even 1 case of air-dried, Giemsa-stained FNA smears. Unfortunately, the only 2 samples that gave no result were from FNA cell blocks.

A report from the Molecular Assay in NSCLC Working Group of American Society of Clinical Oncology notes that “larger tumor samples are generally preferred” for NSCLC molecular assessment, while acknowledging that minimally invasive biopsies such as FNAs are often the only tumor samples available.<sup>21</sup> Despite this, the recommendations from this Working Group highlight further impediments to the utilization of FNAs for this testing. In the preanalytical phase, they state the following: “cytology smears are not acceptable for IHC and FISH”; “at least 3 representative areas should be assessed per tumor section”; “tissue block preferred”; “minimum sample size is ~ 2000 cells for IHC/mutation analysis”; “EGFR sequencing requires high tumor cell content (50%-70% tumor cells)”; “FFPE is standard.” These preanalytical requirements are reasonable, but such recommendations from high-profile National working groups create challenges for the future inclusion of FNA samples into clinical trials and, consequently, into subsequent clinical care.

### KRAS

KRAS mutations are found in approximately 40% of CRCs, and a retrospective review of 5 trials of cetuximab or panitumumab has recently shown that patients whose tumors harbor KRAS mutations will not benefit from therapy utilizing these EGFR-targeting monoclonal antibodies. This has led the American Society of Clinical Oncology to recommend KRAS mutation testing in all CRC patients who are candidates for anti-EGFR antibody therapy.<sup>22</sup> The College of American Pathologists has also

issued a report detailing the appropriate sample types and testing methods.<sup>23</sup> Although much of this testing will be performed on resected primary tumor samples of CRC, metastatic lesions may also be used, which may be more relevant to the cytopathologist. Like many other predictive molecular tests, the approved sample processing methods include only fresh, frozen, or FFPE material. A pathologist must confirm that the sample contains a sufficient number and percentage of tumor cells that is acceptable for the assay being used. Samples that do not contain a sufficiently high percentage of tumor cells may have to be enriched by macrodissecting areas of tumor on slides for DNA extraction.

There is currently no FDA-approved test for KRAS mutation detection, so validated laboratory developed tests performed in CLIA-approved labs must be utilized. Each test may have different sensitivities, and so sample preparation will have to be matched to individual tests. Several of the large CRC clinical trials utilized a test kit (TheraScreen KRAS; DxS, Ltd.) that combines allele-specific PCR with real-time PCR technology for detection of 7 different mutations within codons 12 and 13 of KRAS. This test can purportedly detect 1% of mutant KRAS in a wild-type background. The exclusion of FNA samples processed with traditional alcohol-based fixatives is not due to the quality of the DNA within the fixed sample. In fact, alcohol-based fixatives are significantly better fixatives for DNA than formalin, which can damage DNA.<sup>24,25</sup> Such damage may even result in artifacts that yield false positive mutation detection, particularly when using small amounts of DNA, such as that which might be found in a cell block preparation from an FNA sample.<sup>26</sup> Gaining a better understanding of this type of testing is essential because other gene mutations, such as those from BRAF, may also begin to play an important predictive role in CRC and other tumors, such as papillary thyroid carcinoma.

### Emerging mRNA-Based Profiling Tests

If pathologists are struggling to apply relatively simple tests to samples, such as PCR-based detection of isolated KRAS mutations, imagine the challenges presented by the complex, multi-analyte, and mRNA-based tests that are emerging.<sup>27</sup> The most clinically relevant of these include Oncotype DX (Genomic Health, Inc.) and Mammaprint (Agendia). Oncotype DX is a multi-gene

expression profile test that may be utilized to determine which women with early-stage, lymph node-negative breast carcinoma will benefit from adjuvant chemotherapy. Similarly, Mammaprint is a 70-gene profile that determines the risk for recurrence in early-stage breast cancer patients. These complex tests fall under the FDA heading of an in vitro diagnostic multivariate index assay. To date, single commercial laboratories develop, validate, and exclusively offer such tests because of the strict control necessary to produce valid test results. Neither Oncotype DX nor Mammaprint is validated for FNA samples; Mammaprint is validated for fresh or FFPE tissue and OncoType DX for FFPE tissue only. This is despite the finding that FNAs might actually provide a higher quality and quantity of mRNA from some samples than an FFPE core biopsy. Because the indications for these tests are currently for early-stage breast cancer, which is rarely biopsied via FNA in the United States, it is unlikely that these commercial suppliers will invest in the validation required for FNA samples. This unfortunately sets another precedent for the exclusion of FNA samples from an important class of predictive molecular testing.

### ***Impediments to FNA-Based Predictive Tests***

#### **FNA Biospecimen Issues**

There is increasing awareness that the quality of biospecimens, such as FNAs, has a profound influence on downstream molecular diagnostic test results. As stated by the newly created National Cancer Institute Office of Biorepositories and Biospecimen Research: "The reliability of molecular data derived from . . . new analysis platforms is dependent on the quality and consistency of biospecimens being analyzed."<sup>28</sup> Unfortunately, there are very few studies that rigorously compare the cellular composition of FNA samples with the quantity and quality of the desired analyte (DNA, mRNA, or protein) or the robustness of the biomarker test utilizing the sample. As has been discussed in the section on EGFR mutation testing, sample size and heterogeneity may have a profound effect on the downstream molecular test results. Centeno et al utilized "benchtop" FNAs on a relatively small and diverse set of surgically excised tumors to explore the role of needle gauge and excursion number on the sample composition,

RNA yield, and suitability for microarray analysis.<sup>29</sup> They found that the samples comprised 47%-98% tumor cells, yielded cell counts that ranged from 10,000-1 million cells per pass, and 0.92 ug-12 ug of total RNA. A multidisciplinary group at The University of Texas M. D. Anderson Cancer Center has extensively used FNA samples of primary breast cancers for gene expression profiling. Such FNA samples typically contained 80% tumor cells and yielded a mean of 3.6 ug of total RNA, which was frequently suitable for microarray analysis.<sup>30</sup> Far more studies of this nature are necessary to elucidate the molecular composition and biomolecule integrity of FNA samples to consistently connect FNA samples to downstream molecular diagnostics.

In addition to the sample composition, the specimen preanalytical handling and processing methods have a profound impact on biomolecules within the sample and the quality of molecular tests based upon these molecules. As previously discussed, variability in the FFPE fixation and processing methods has historically confounded HER2 IHC testing. Unfortunately, FNA samples are exposed to an even greater variety of cytopreparation methods than resected tissue. For example, samples may be fixed in methanol, ethanol, isopropanol, formalin, or some combination of these. Samples may be processed as direct smears, cytopins, monolayer preparations, or cell blocks. Historically, cytopreparation methods have been geared toward optimization of cytomorphology to facilitate accurate microscopic diagnosis. Even Dr. Papanicolaou recognized the importance of cytopreparation and preanalytical variability on the quality of cytologic diagnoses. In the 1940s, when challenged by an individual who claimed that cytologic diagnoses were not possible, Dr. Papanicolaou reviewed that individual's prepared samples and stated that he agreed: "I couldn't make a cytologic diagnosis from such poor technical preparations."<sup>31</sup> The science of cytopreparation was furthered in the 1960s and 1970s by individuals like Dr. John K. Frost and Gary Gill at Johns Hopkins, culminating in the foundation of our currently utilized techniques.<sup>32</sup> We now need a new generation of cytotechnologists and cytopathologists who are dedicated to optimizing and standardizing FNA sample preparation methods not only for cytomorphology, but also to preserve biomolecular integrity to enable seamless integration into molecular testing.

**Predictive Test Development**

When considering the impediments to FNA-based biomarker tests, it is useful to put them in the context of the developmental pathways recently proposed by the NCI’s Translational Working Group, specifically the Developmental Pathway for Biospecimen-Based Assessment Modalities.<sup>33</sup> The first step in this pathway is the definition of the clinical goals that are driving the biomarker development. One obvious goal is the development of prognostic tests to determine outcome and tests predictive of response to targeted therapies. This will require cytopathologists to be significantly engaged with the clinical and therapeutic aspects of cancer care to be knowledgeable partners in this process (Tables 2 and 3). Once specific biomarker goals have been defined, a credentialing step is necessary to determine which fundamental research observations yield candidate biomarkers with clinical potential.

In designing and conducting studies to determine the diagnostic accuracy of any test, guidelines developed by the Standards for Reporting of Diagnostic Accuracy steering committee should be followed.<sup>34</sup> The next step in the developmental pathway is the identification of technical challenges that require new enabling technologies. This might involve the development of novel FNA processing devices that are standardized and optimized for molecular testing of FNA samples. In addition, to utilize specimens obtained from minimally invasive biopsies, such as FNAs, analytical technologies that are highly sensitive and permit multiplexing will be required.

To move credentialed biomarkers forward, an assessment modality, or biomarker assay, will then need to be developed. Ideally, this assay development will be aimed toward an assay that has the necessary performance characteristics to implement in a clinical laboratory, not

**Table 2.** Glossary of Terms Relevant to Molecularly Targeted Agents and Biomarkers

Term	Definition
Adjuvant therapy	Treatment given after the primary treatment to increase the chances of a cure.
Analyte	The part of the sample that a test is designed to find or measure.
Biomarker	A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.
In vitro diagnostic multivariate index assay (IVDMIA)	An IVDMIA employs data, derived in part from one or more in vitro assays and an algorithm that usually, but not necessarily, runs on software, to generate a result that diagnoses a disease or condition or is used in the cure, mitigation, treatment, or prevention of disease.
Laboratory-developed test	A test developed by a clinical laboratory for use only by that laboratory.
Molecularly targeted therapy	In cancer treatment, substances that kill cancer cells by targeting key molecules involved in cancer cell growth.
Neoadjuvant therapy	Treatment given as a first step to shrink a tumor before the main treatment, usually surgery, is given.
Predictive factor	A condition or finding that can be used to help predict whether a person’s cancer will respond to a specific treatment.
Prognostic factor	A situation or condition, or a characteristic of a patient, which can be used to estimate the chance of recovery from a disease or the chance of the disease recurring.

Sources: <http://www.cancer.gov/dictionary>; <http://www.fda.gov>

**Table 3.** Resources Relevant to Molecularly Targeted Agents and Biomarkers

Topic	Website
National Cancer Institute drug dictionary	<a href="http://www.cancer.gov/drugdictionary/">http://www.cancer.gov/drugdictionary/</a>
FDA Center for Drug Evaluation and Research Office of Oncology Drug Products	<a href="http://www.fda.gov/AboutFDA/CentersOffices/CDER/">http://www.fda.gov/AboutFDA/CentersOffices/CDER/</a>
FDA in vitro diagnostics	<a href="http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/default.htm">http://www.fda.gov/MedicalDevices/ ProductsandMedicalProcedures/InVitroDiagnostics/default.htm</a>
National Cancer Institute Office of Biorepositories and Biospecimen Research	<a href="http://biospecimens.cancer.gov/">http://biospecimens.cancer.gov/</a>
Oncology clinical practice guidelines	<a href="http://www.nccn.org">http://www.nccn.org</a> ; <a href="http://www.asco.org">http://www.asco.org</a>

just a research setting. For traditional biomarkers, this often utilizes archival specimens for retrospective studies. It is the molecular diagnostics industry that oftentimes has the necessary resources and expertise to facilitate such complex method development and validation.<sup>35</sup> Once this analytical validation is complete, the biomarkers are ready for clinical validation in clinical trials. It is important to note that while a biomarker test result might make intuitive sense as a predictor of response, the ultimate proof resides in clinical validation. It is also important to note that a biomarker that is a clinically validated predictor in one tumor type or for a given drug may not be predictive in another tumor type, or for another drug of the same class. Finally, different biomarker tests might have very different performances so each biomarker test and clinical indication needs to be individually validated.

### ***Opportunities for the Future***

One clear entry point for FNA-based predictive tests for cancer is in the advanced disease setting. The first indications for most new molecularly targeted agents are typically in locally advanced or metastatic disease. Such patients are often not surgical candidates, and so tissue access for molecular characterization through a minimally invasive approach, such as FNA is ideal. Many cancer patients present with such advanced disease, including approximately 50% of lung cancers, 20% of colon cancers, and 50% of pancreatic cancers.<sup>36</sup> Recurrent disease in lymph nodes or liver is also an obvious target for FNA sampling. In metastatic breast cancer, it has been demonstrated that expression of estrogen/progesterone receptors and EGFR may be significantly different in metastatic lesions than in the primary tumor, which may impact therapeutic decisions.<sup>37</sup> In addition to this advanced disease setting, patients receiving neoadjuvant therapy may be good candidates for molecular testing of FNA samples. Such patients may demonstrate a complete pathologic response to the therapy upon surgical resection, with minimal or no residual tumor, placing increased importance on the molecular characterization of the initial biopsy. Finally, the minimally invasive nature of FNA biopsies opens the opportunity for serial sampling of an individual's tumor, over time, to assess the efficacy of a targeted therapy through molecular characterization, or to identify molecular markers of resistance.

FNA cytology needs to move beyond its perceived second-class status to histologic sampling and establish itself as an independent diagnostic modality to guide therapy. This will be accomplished by coupling cytomorphology with molecular characterization. Such independence will also fuel creative new uses of FNA samples. For example, new techniques may be developed to exploit the finding that FNAs can procure a suspension of tumor cells that are alive. This has been utilized to facilitate flow cytometric phenotyping of lymphomas, but it also opens the door for the analysis of "ex vivo biomarkers," ie, biomarkers that are evoked from live tumor cells outside the body to reveal functional information about the tumor. This has already been applied in the research setting to characterize lymphoma and leukemia, as well as FNA samples from solid tumors.<sup>38,39</sup>

If cytopathologists fail to rise to the challenge of molecular cytopathology, the information necessary to select targeted therapy will be acquired from sources other than FNAs. For example, circulating tumor cells can already provide prognostic information about metastatic breast, colorectal, and prostate cancers, and they can potentially yield information about EGFR mutation status of lung cancers.<sup>40-43</sup> The field of proteomics promises to identify protein markers in peripheral blood that serve as surrogates for tissue-based biomarkers.<sup>44</sup> And advances in molecular imaging are increasingly producing noninvasive radiologic tests to monitor tumor function and response to therapy.<sup>45</sup> Cytopathology is indeed both an art and a science; however, we must increasingly strive to become more scientific if we are to provide the high-quality, value-added results, which are required to participate in the current age of targeted cancer therapy.

### ***Conflict of Interest Disclosures***

Dr. Clark is entitled to a share of equity as a founder of BioMarker Strategies, LLC. He currently is an officer for the company. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

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