

# Biopsies in the Age of Precision Medicine

The crossroads of molecular biology and medical imaging.

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Our understanding of the cellular, subcellular, genetic, and molecular basis of disease has rapidly advanced over the past 50 years. What we once regarded as a single disease may actually represent a common phenotypic manifestation of a variety of underlying microscopic derangements. In parallel, we have developed medical imaging technologies capable of peering deep inside the body with ever-increasing structural resolution. Radiologists continue to examine the body through a telescope rather than a microscope, and medical imaging depicts the gross structural manifestations of disease without revealing the fine microscopic features that define each entity within the spectrum of human conditions.

It is precisely these cellular, genetic, and molecular features of disease that offer opportunities for targeted treatments with the hope of improved efficacy and fewer side effects. Decades ago, a patient with an apical lung mass might have been surgically biopsied and then given a diagnosis of lung cancer with treatment based on size, location, and stage of the disease. Now, the same mass might be percutaneously biopsied and diagnosed as lung adenocarcinoma with high expression of an epidermal growth factor receptor and a critical KRAS mutation.

Today, the treatment plan might include drugs that target the unique molecular characteristics of the tumor. Drilling down to identify the molecular signatures of disease reveals subpopulations of patients with conditions that are susceptible to targeted treatments. Knowing which patients have a disease that may respond to these new therapies helps physicians prescribe these drugs specifically to the patients they are designed to help. Once we are able to make these microscopic phenotypes visible on clinical imaging examinations, we will have a noninvasive way to make more refined diagnoses and monitor disease progression and treatment response over time. Until then, there is no substitute for the microscope.

## BIOPSIES REVEAL WHAT IMAGING DOESN'T

Biopsies provide key structural, cellular, molecular, and genetic information. They can also help guide staging, inform estimates of prognosis, determine which tumors may be susceptible to targeted treatments, and identify possible familial risk. Biopsy continues to be the gold standard for diagnosis and disease characterization for both clinical and research applications and is likely to continue to serve as such for some time. Even as molecular imaging modalities capable of detecting specific derangements emerge, it is likely to be decades before they can provide the amount of information and level of detail that biopsies can give regarding the combination of various molecular changes and microscopic structural aberrations present.

Rather than supplanting the role of biopsy, this age of targeted therapies is generating new indications for biopsy. After establishing the initial diagnosis, there is an increasing need for serial tumor samples in order to monitor disease progression and response to treatment over time. Obtaining a sample of recurrent disease (as in lymphoma) or of treatment-resistant areas of disease constitute other opportunities to precisely tailor the treatment to tumors as they devolve. Despite these benefits, biopsy is not without its limitations. Recognizing and understanding these limitations is essential for obtaining the highest-quality tissue sample and giving patients the best chance at an effective treatment.

## ROLE OF INTERVENTIONAL RADIOLOGISTS IN THE PRECISION MEDICINE INITIATIVE

The promise of precisely matching a patient to the appropriate treatment led to the concept of “personalized medicine.” Also known as “precision medicine,” this new health care strategy involves selecting the optimal treatment based on a patient’s individual



**Figure 1.** The National Cancer Institute's infographic depicts the goal of precision medicine to target cancer treatment to a patient's individual genetic aberrations. Reproduced from the National Cancer Institute. <http://www.cancer.gov/multimedia/infographics/precision-medicine/nci-precision-medicine-infographic>. Accessed July 26, 2016.

characteristics, including genetic, molecular, and cellular features of the disease (Figure 1).<sup>1</sup> Some of these features cut across organ systems and other traditional diagnostic boundaries, thereby opening up new applications for available treatments. All of this hinges on accurate tissue biopsy and analysis. Today, very few biopsies are performed surgically; the vast majority are performed by interventional radiologists (IRs) who are uniquely trained to safely and accurately sample tissue percutaneously. These skills make IRs the ideal

physicians to perform biopsies for precision medicine applications.

On January 30, 2015, President Barack Obama announced the National Institutes of Health's (NIH's) \$215 million Precision Medicine Initiative in his State of the Union address.<sup>2</sup> In his speech, President Obama stated,

Doctors have always recognized that every patient is unique, and doctors have always tried to tailor their treatments as best they can to individuals. You can match a blood transfusion to a blood type—that was an important discovery. What if matching a cancer cure to our genetic code was just as easy, just as standard?

The Precision Medicine Initiative is sponsoring a study called the National Cancer Institute-Molecular Analysis for Therapy Choice, known as NCI-MATCH or study EAY131 (Figure 2).<sup>3</sup> The aim of this phase 2 trial is to determine whether treating tumors with drugs designed to target their specific genetic abnormalities would be effective, regardless of the cancer type. NCI-MATCH rapidly accrued nearly 800 patients, but infrastructure issues, including problems with biopsy tissue quality, forced the NIH to pause the trial.<sup>4</sup> Up to one in five biopsy samples were inadequate for genetic analysis, thereby disqualifying these patients from the trial.<sup>5</sup> The fact that IRs were performing most of the biopsies was the focus of a recent article and presents an opportunity for self-reflection.<sup>5</sup>

## THE CHALLENGE OF PROCURING PRECISION BIOPSIES

Given the advanced skills common to IRs, it is hard to believe that the issues with the samples cannot be circumvented with education. According to the article, the reason that some of the samples were nondiagnostic is that they contained insufficient tumor cells for analysis—in some cases, fewer than the requested four core samples were submitted, and some of the tissue was of poor quality.<sup>5</sup> It may be that the samples would have been adequate for gross anatomic diagnosis, but more tissue is needed for the advanced genetic analyses required for the study. Because each sample incurs risk, there is an inherent tendency to take the minimum, but the benefits of biopsy may outweigh these risks. Indeed, patients enrolling in the NCI-MATCH trial had failed conventional therapies, so the trial may have constituted their last chance at an effective treatment.<sup>5</sup>

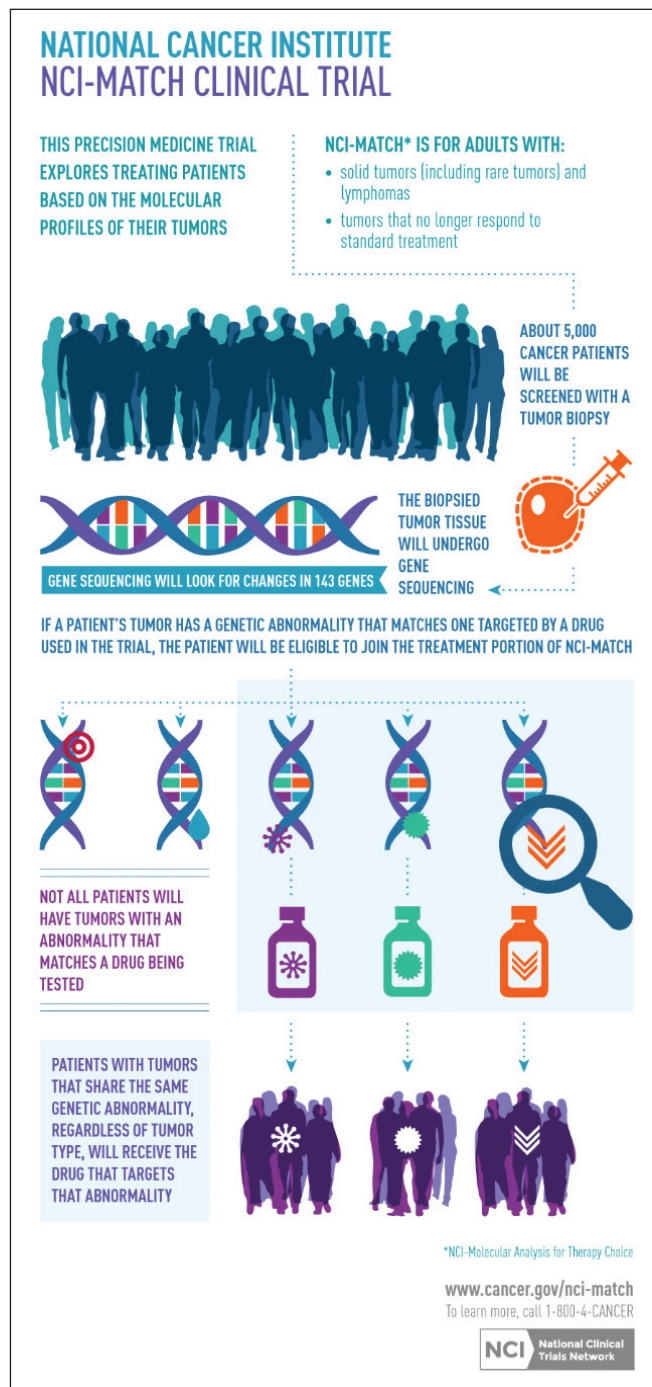
The quality of the biopsy sample can also suffer when too much necrotic tissue is included, and the reported paucity of live cells in some of the samples suggests that

this may have been an issue. The general tendency to sample the center of mass may increase the likelihood for obtaining necrotic tissue (Figure 3A), as tumors can outstrip their blood supply as they grow. Finally, placing the tissue samples in the wrong solution or otherwise handling the tissues improperly can also degrade their quality or interfere with analysis.

Obtaining a quality biopsy sample can be challenging for both technical and biological reasons. Two of the most significant features that can make a tumor difficult or even risky to biopsy are its size and location. Small tumors deep inside the body require greater precision when performing a biopsy from a percutaneous approach than large or superficial tumors. If the tumor is surrounded by bone, bowel, lung, or arteries, a direct path may be impractical or risky, and more oblique or even curved approaches become necessary.

Poor tumor conspicuity on imaging due to weak inherent contrast differentiation from surrounding tissue can also make it difficult to obtain a quality sample or even aim for a particular aspect of the tumor. Suboptimal image quality due to technical limitations or patient body habitus can also make tumors difficult to see. Motion, whether voluntary or involuntary, adds another level of difficulty, especially when guided by CT or MRI. Biopsies are riskier and more challenging in patients who cannot remain still or when the biopsy is performed in areas affected by respiratory motion or bowel peristalsis. Apart from these technical factors, there are also features inherent to a tumor that can make it challenging to obtain a sample that accurately reflects its nature.

Tumors are both heterogeneous and dynamic. There is heterogeneity within a mass, across masses within a patient, and among patients with the same diagnosis.<sup>6,7</sup> In order to provide the correct diagnosis, the biopsy must be performed on the representative tissue. For a heterogeneous mass, sample bias could have significant implications on the diagnosis and treatment. Basing treatment decisions on a biopsy of an indolent portion of a mass that also harbors more aggressive areas could lead to treatment failure. In a patient with more than one mass, what constitutes representative tissue depends on whether the goal is to establish a primary diagnosis or identify metastatic disease, as the tumor profile can vary between these. Differences in tissue cellularity across a mass with solid, cystic, and fibrotic components can affect



**Figure 2.** This infographic by the National Cancer Institute depicts the design of the NCI-MATCH trial. Patients in the NCI-MATCH trial first undergo tumor biopsy and genetic analysis, then they are stratified to a number of different drugs that target their specific genetic abnormality. Some patients will have genetic abnormalities for which there is no drug available. Reproduced from the National Cancer Institute. <http://www.cancer.gov/multimedia/infographics/nci-match/nci-match-clinical-trial-infographic>. Accessed August 22, 2016.



sample quality, depending on the region biopsied. Attention to the inherent structural heterogeneity of tumors while obtaining a sample is an important step toward improving yield and quality.

### GETTING BETTER SAMPLES

Unfortunately, there are very few data regarding how to obtain a quality biopsy sample for cellular, molecular, and genetic analysis. Molecular imaging tools to help elucidate the salient areas for biopsy are not readily available at most institutions. However, knowledge about tumor growth and tissue processing may help increase the yield.<sup>8</sup>

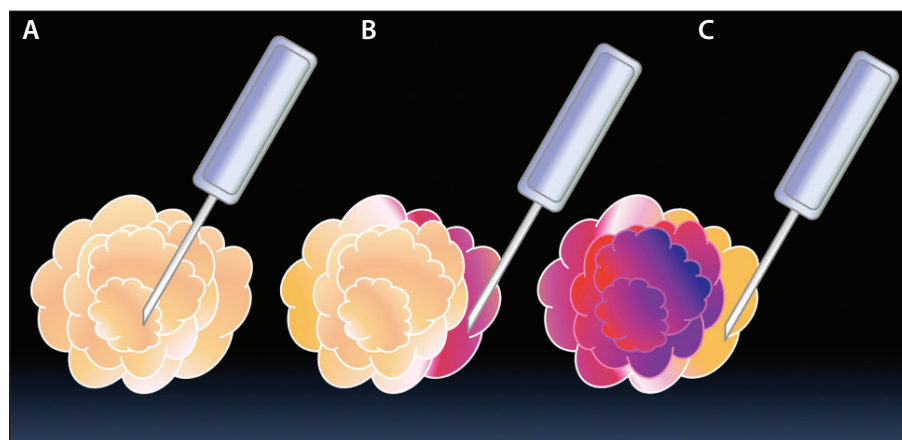
The technical challenges of biopsy can largely be circumvented with the proper imaging equipment, operator skills, and tools. The use of high-quality ultrasound imparts even greater control to the operator by providing real-time information about features of the mass and its location relative to surrounding vascular and nonvascular structures, even in the setting of motion. IRs are uniquely skilled at using ultrasound for guiding complex percutaneous interventions. Advances in biopsy needle technology promise to make it easier to target areas of concern and obtain sufficient tissue for diagnosis.<sup>8</sup> Patient motion can be mitigated through coaching and sedation, and bowel peristalsis can be diminished with glucagon.

Overcoming some of the biological challenges of tumor biopsy can be less straightforward. For a heterogeneous mass, obtaining a sample of the most aggressive parts of the mass may trigger the treatment with the greatest opportunity to control the cancer. Aiming for new lesions and solid, disorganized areas (especially if they are encroaching on adjacent structures) may increase the chance of obtaining a sample of more devolved and aggressive areas that would be important for guiding treatment. Biopsy of multiple different areas or even more than one mass may help mitigate (but not eliminate) some of the differences encountered due to tumor heterogeneity. Representative tissue must contain a certain number of viable cells, so one should

avoid paucicellular tissues including fibrosis/scarring, cystic change, and necrosis. Color Doppler can help identify areas of living tissue in real time. Alternatively, obtaining a sample of the tumor's periphery may help circumvent central necrotic areas (Figure 3). Evaluation of previous positron emission tomography (PET) exams can also help identify which areas are alive (fluorodeoxyglucose [FDG] avid) and most active (degree of avidity).

It is also critical to obtain sufficient tissue for advanced testing. Because the tissue volume requirements can vary widely among tests (and even decrease as tests become more efficient), it is advisable to consult the ordering physicians and pathologist to determine how much tissue is needed and how it should be submitted. Core biopsies have the advantage over fine-needle aspirations of obtaining more tissue and preserving some of the tissue architecture, whereas fine-needle aspirations provide cellular aspirates. Larger-gauge biopsies provide more information but come at a greater bleeding risk. Using a coaxial approach and ablating the tract with < 2 mL of absolute ethanol induces coagulation necrosis in the tract that not only stops the bleeding but also mitigates the very small risk of tumor seeding.

Finally, it is critical to place the samples in the appropriate solution (a cell culture medium if the cells are to be grown or subjected to flow cytometry or formalin if they are to be analyzed structurally). If in doubt, samples may be placed into culture medium and then moved to formalin, but samples in formalin become fixed and cannot be grown or analyzed by flow cytometry. In the



**Figure 3.** Strategies for performing an image-guided biopsy of a tumor may change with the advent of molecular imaging. When performing a biopsy based on structural imaging (A), such as CT, MRI, or ultrasound, it is common to aim for the bulk or the center of the mass, although the center is more likely to contain necrotic tissue. Biopsies guided by molecular imaging (B, C) would allow IRs to target areas that enhance with a targeted contrast agent (B, depicted in magenta). Alternatively, areas that do not enhance or are not responding to targeted therapy (C) could be sampled for better characterization.

rare instances that fresh frozen tissue is needed, the sample must be collected in person and promptly flash frozen. Understanding the purpose of the sample and how it will be processed will enable the IR to choose the correct site, obtain sufficient tissue, and submit it in the proper solution for analysis.

## BIOPSIES AND BEYOND

Innovations in imaging, software, and needle technology have already begun to improve biopsy accuracy and yield. Fusion imaging is most accessible and allows FDG-PET data to be combined with the structural data from cross-sectional imaging and then coregistered with biopsy imaging (eg, CT, ultrasound) in order to permit targeting of FDG-avid areas. With the emergence of new, targeted PET radiotracers (as for prostate and breast cancer), there will be new opportunities for fused imaging. New molecular contrast agents for MRI that concentrate in target tissues may also help direct biopsy (either MR-guided or through fused images) or sometimes even supplant it. Depending on the clinical question, IRs could choose to aim for areas of molecular contrast agent uptake (Figure 3B) or areas within an otherwise enhancing mass that do not take up the targeted agent (Figure 3C).

Another promising technology is contrast-enhanced ultrasound, which recently received US Food and Drug Administration approval for characterizing liver lesions. Ultrasound has the benefit of allowing real-time imaging, and the microbubbles afford perfusion and washout information that can improve the accuracy of a biopsy. Microbubbles also have the potential to be decorated with molecules to enhance their localization to target tissues, and they can also be filled with diagnostic or therapeutic payloads that can be released upon uptake or with the application of certain ultrasonic frequencies. Biopsies could also be targeted based on information from radiogenomics, in which features of tumors on medical imaging exams correlate with gene expression.

Finally, liquid biopsies detect circulating tumor cells or tumor-associated proteins in the blood.<sup>9</sup> Although touted as an alternative to tissue biopsies for cancer, liquid biopsies are likely to serve a complementary role by providing additional information regarding disease progression or response to therapy over time once the initial diagnosis is established. These emerging technologies all stand to improve the accuracy of biopsy by revealing particular areas of interest to sample or by providing additional information that could refine the diagnosis further.

## CONCLUSION

Even as better structural and molecular imaging techniques emerge, biopsy is not likely to become obsolete in the near future. Improved diagnostic yield will come from educating IRs on tissue requirements for precision medicine purposes, developing smarter tools for biopsy and image guidance, and combining complementary data. The skills that IRs use to obtain a high-quality biopsy sample from a technically difficult lesion for diagnosis are the same that are needed to precisely place a probe for complete thermal ablation, a localizing needle to aid in surgical excision, or fiducials for radiation therapy. When the technology is ready, these same skills will enable IRs to perform the initial tumor biopsy, then leave a biosensing microchip, a dose of oncolytic virus, or engineered stem cells to defeat the cancer.

Molecular imaging remains on the horizon, and even farther down the road is the potential to capture the complexity of a tumor in an imaging exam, thereby rendering biopsies unnecessary. Until then, biopsies are the link between biology and imaging, and they are the portal to theragnostic approaches that unify diagnosis and treatment in a single procedure. Medicine is at a crossroads, and IRs are poised to stand at the intersection and usher in this new age of precision medicine. ■

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