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Personalizing Precision Oncology: From Light Microscope to Molecular Microscope

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The paradigm of cancer therapy is evolving from nonspecific cytotoxic drug therapy based on histologic diagnosis alone to one that aims to deliver the “right drug to the right patient at the right time.”¹ With the advent of precision oncology fueled by clinical next-generation sequencing (NGS) has come an exponential growth in the use of genomically targeted therapy and immunotherapy. NGS is most commonly used in advanced, metastatic cancer and in rare cancers in which conventional histologic diagnosis may be challenging and for which there are few defined standard of care therapies. Because every patient’s sequence is unique, a precise molecular diagnosis and a customized treatment plan are needed when standard therapies become ineffective. Clinical NGS makes this feasible by enabling pathologists to supplement the histologic diagnosis with molecular data that, in turn, help clinicians develop a rational therapeutic strategy. For example, identifying a particular genomic aberration can open up new avenues of therapy, such as enrollment in a tissue-agnostic basket trial.

When clinical trials are not an option, a therapy targeting a specific alteration may be used off-label if the treatment is approved in another tumor type for the same alteration. The decision tree for matched therapies outside clinical trials is often challenging, and the benefits versus risks of treatment need to be carefully weighed. In this issue of *JNCCN*, Tsai et al² report an interesting case of metastatic undifferentiated carcinoma with clear cell change harboring multiple mutations, including a *PTCH1* A925fs*6, W948* mutation refractory to hedgehog inhibition. This patient was successfully treated using immune checkpoint blockade therapy based on a commercially available comprehensive genomic panel. This case highlights several issues related to the contemporary, multidisciplinary management of cancer from diagnosis to therapy.

One of the major challenges with respect to rare cancers or rare variants of common cancers is arriving at the correct pathological diagnosis. The diagnosis and classification of cancer have traditionally been rendered by pathologists who interpret the cytologic and architectural patterns of cells and stroma from microscopic examination of tissue sections stained by hematoxylin and eosin. Overlapping histologic features sometimes generate a differential diagnosis—in the report by Tsai et al, a clear cell neoplasm that could have been diagnosed as squamous cell carcinoma, basal cell carcinoma (BCC), or adnexal clear cell carcinoma—that may require clinical correlation and ancillary studies to resolve. Standard immunohistochemical studies may also be of limited utility, as in this case. NGS in this setting serves as a molecular microscope, enabling pathologists to reach a molecular diagnosis that can augment the histologic diagnosis. Also, the addition of genomic profiling to management of rare cancers adds a potential line of therapy for cancers that have little or no standard of care.^{3,4}

In the report by Tsai et al,² the sequencing results that showed multiple mutations, including a *PTCH1* mutation, were a key feature in identifying the tumor as a BCC of variant histologic subtype. Without a precise diagnosis, a treatment plan is difficult to formulate. The diagnosis of BCC with a *PTCH1* mutation helped

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guide targeted therapy with a sonic hedgehog pathway inhibitor, whereas the patient's previous recurrences were diagnosed as "BCC" and "poorly differentiated clear cell carcinoma" without molecular annotation and were treated with surgery and radiation therapy. The tumor harbored 22 mutations in 18 genes, several of them driver aberrations. This presents a conundrum of how to treat a disease with multiple mutations, including several actionable oncogenic alterations. The patient was initially treated with targeted therapy, to no avail. He was subsequently treated successfully with immune checkpoint blockade therapy based on a high number of pathogenic alterations, a surrogate for tumor mutational burden (TMB), and the presence of an inactivating *MLH1* alteration (a marker for mismatch repair deficiency [dMMR]/microsatellite instability-high [MSI-H] tumor).

Despite the patient's *PTCH1* mutation, the tumor did not respond to a hedgehog inhibitor. In this context, the *PTCH1* mutation is likely a red herring in the presence of multiple actionable alterations in a patient with a hypermutated tumor. Because hypermutation is increasingly correlated with response to immune checkpoint inhibitor therapy, it has been the subject of large studies to decipher the resistance and response mechanisms involved.

Clearly, greater insight into the dynamics of hypermutation is required.⁵ First, the driver aberrations in these sequences must be decoded. These aberrations can be baffling, given the large number of passenger variants obfuscating the driver variants. Moreover, many of the "somatic mutations" in hypermutated cancer may not have functional significance.⁵ Second, any of these aberrations could be early events and the cancer genome could have plausibly undergone evolution by accumulating additional drivers, thereby rendering the inciting mutations as passenger mutations. This mutagenic trajectory could leave an imprint on the genome from a simple tumor evolving into a complex tumor. Third, no consensus is available regarding the definition of "hypermutation" with respect to TMB, because different groups have proposed divergent thresholds for what constitutes a high versus a low TMB.

Notably, this patient's tumor was PD-L1–negative by immunohistochemistry. Although PD-L1 (B7-H1) immunostaining has emerged as a biomarker for response

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Figure 1. Biomarkers of response to immunotherapy are overlapping and form a spectrum. Abbreviations: MSI-H, microsatellite instability-high; TIL, tumor-infiltrating lymphocytes; TMB, tumor mutational burden.

to immunotherapy, this biomarker is imprecise given the tissue heterogeneity, different antibodies used for detection, variable sample preparation and methodology for interpretation, and dynamic changes over time. The response rates to immunotherapies range between 36% and 100% for PD-L1–positive tumors compared with 0% to 17% for PD-L1–negative tumors.^{6,7} In addition to PD-L1 and TMB, NGS testing can also suggest DNA dMMR. This finding has major implications for therapy, because the FDA recently approved pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic MSI-H or dMMR solid tumors that have progressed after previous therapies.^{8–10}

MSI-H or dMMR is identified by mutation or methylation status of 4 genes: *MLH1*, *MSH2*, *MSH6*, and *PMS2*.⁹ Microsatellite biomarker status is evaluated using a commercially available assay for mutational assessment of 5 diagnostic microsatellite sequences; a pathology specimen is noted as MSI-H if at least 2 of 5 microsatellites harbor mutations. NGS or immunohistochemistry for MMR proteins may be also used to arrive at the dMMR status. In this case, the patient had an inactivating *MLH1* aberration conferring the dMMR status and making him potentially a good candidate for pembrolizumab therapy. The approval of pembrolizumab represents a major milestone in precision oncology because it is the first cancer treatment approved by the FDA for

a tissue-agnostic indication rather than based on the tumor's histology or primary site of origin.⁸

Other immunotherapy biomarkers have emerged that are the subject of active translational research in clinical trials, including tumor-infiltrating lymphocytes, *PD-L1* gene amplification, interferon signature indicating a T-cell–inflamed phenotype, mutation-associated neoantigens, microbiome repertoire, and other genomic defects.^{7,10} These biomarkers are not mutually exclusive but rather represent a spectrum and could be overlapping (Figure 1). In addition to NGS, molecular profiling may be improved by gene expression profiling,¹¹ transcriptomics, comprehensive immune profiling, and microbiome studies.

As shown in the report by Tsai et al,² metastatic tumors are characterized by molecular heterogeneity and genomic complexity, making their treatment one of the major challenges to the current precision medicine approach. Genomically targeted therapy and immunotherapy represent 2 modern pillars of oncology that are here to stay. However, biomarkers of resistance and response to these agents are urgently needed given that these therapies are not always effective and are associated with nonnegligible toxicities and cost. Molecular characterization of tumors is increasingly feasible in a clinical setting and can potentially add another line of therapy as we untangle the genetic underpinnings of cancers from the light microscope to the molecular microscope.

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