BRAF INHIBITOR PROGRESS AND PITFALLS

"It's often difficult to define how personal is 'personalized,'" said Hensin Tsao, MD, PhD, associate professor of dermatology at Harvard Medical School and director of the MGH Melanoma and Pigmented Lesion Center at Massachusetts General Hospital. "Personalizing medicine goes beyond the genetic dissertation of what the tumor has; it's much more about finding the precise signature that comes with an action which will, with high probability, influence an outcome. Technology has allowed us to identify a broad spectrum of markers that helps us define the tumor in the individual with precision. That's the personalization part. Now we have to be able to balance that with the medicine part."

The investigation of the V600E BRAF mutation and subsequent development of drugs that target the mutation illustrate both the benefits and challenges inherent in personalized medicine, and selective inhibitors in particular. BRAF is a version of RAF in the MAP kinase signaling pathway of RAS-RAF-MEK-ERK. The V600E BRAF mutation was first identified as an oncogene in melanoma in 2002, and is found in about 50 percent of primary and metastatic melanoma tumors. "In the early days, we didn't have potential drugs that we could give patients, but now the first two such drugs have gone through phase III clinical testing as single therapy for patients whose melanoma has the BRAF mutation," said Keith T. Flaherty, MD, associate professor in the department of medicine at Harvard Medical School and director of developmental therapeutics at the Massachusetts General Hospital Cancer Center. "The results are quite clear: You can get a very reliable, reproducible early impact on advanced metastatic tumor in 90 percent of patients treated, to a varying degree."

In August 2011, the Food and Drug Administration approved vemurafenib as the first targeted genetic therapy approved for melanoma, along with a diagnostic test to determine if a patient's tumor has the V600E mutation (see "Targeted therapies take aim at skin cancer," www.aad.org/dermatology-world/monthly-archives/2012/march/targeted-therapies-take-aim-at-skin-cancer). A year later, the manufacturer of the second BRAF inhibitor, dabrafenib, and an MEK inhibitor, trametinib, submitted new drug applications for those agents to the FDA. In the year since the approval of vemurafenib, "it seems that it's been fairly rapidly adopted as a treatment standard for patients with the BRAF mutation," Dr. Flaherty said. Testing for the mutation "seems to be happening not only in the big hospital-based practices but in private practice as well. It's certainly close to being universally adopted."

In an abstract updating results of the BRIM-3 trial, which compared vemurafenib to another therapy, submitted to the 2012 annual meeting of the American Society of Clinical Oncology, Chapman et al. reported that median overall survival rates in previously untreated patients with unresectable Stage IIIC or IV melanoma were 13.2 months with vemurafenib and 9.6 months with dacarbazine. "Some patients maintain benefits for two or three years," noted Dr. Flaherty, but then, "as might be anticipated, over time many patients develop resistance to single-agent therapy because these are genetically advanced tumors that have multiple aberrations other than BRAF."

Personalized medicine has to be **precise and it has to be actionable**.

Dr. Tsao remarked that resistance could theoretically take the form of "multiple different metastases in the lung, all taking different routes. Then you don't know what to do." A better approach than single-agent therapy, he suggested, might be "giving cocktails up front based on what you think the most likely mechanisms of proliferation, survival, and resistance. For now, one obvious therapeutic pathway suppresses the cell's reliance on BRAF(V600E)-based signaling. Going forward, maybe you want to look at a few markers, and they may not even be mutational — they could be levels of protein, levels of gene X, Y, whatever it is. Because once you start chasing resistance mechanisms, it may be very hard down the line to get them all." Dr. Flaherty said he has focused his efforts on finding alternative therapies targeting other points in the pathway, to be able to suppress the emergence of metastasis or treat it once it manifests. One strategy involves targeting MEK, the point immediately downstream of RAF in the MAP kinase pathway. In a phase 1 and 2 trial combining the BRAF inhibitor dabrafenib with the MEK inhibitor trametinib, published in the *New England Journal of Medicine* (2012;367(18):1694-703), Dr. Flaherty's team found that median progression-free survival was 9.4 months in the group of patients receiving both drugs, as compared with 5.8 months in the group receiving only dabrafenib.

Another approach to treating advanced melanoma, Dr. Tsao pointed out, is "combining

molecular control with immune control. Now those two large areas of research are beginning to converge, the idea being that you can have very quick, early control that almost everyone will experience with a BRAF inhibitor, and combine that with longer-term immune control of the tumor."

FINDING THE SWEET SPOT

The difficulty in finding agents effective against the NRAS mutation, also common in melanoma, illustrates the current limitations of personalized medicine, Dr. Tsao said. "We've known about NRAS for two decades; it was one of the first oncogenic mutations discovered in melanoma," he said. "But RAS is not a kinase, it's an activated G protein, and it's hard to develop agents against it." Nevertheless, researchers continue to target the NRAS mutation, Dr. Flaherty said, pointing to an early trial of combined pharmacological inhibition of MEK and CDK4 in mice, published in *Nature Medicine* (2012;18(10):1503-10).

Dr. Tsao views the "engine of personalized medicine" as "expanding your mutational and genetic landscape but finding molecular targets that are druggable," and defines the intersection of the two as "the sweet spot in that marriage. Personalized medicine has to be precise and it has to be actionable." At the same time, "you don't want to be so personalized that there's a very small market for what you do. So I think testing these regimens is a challenge down the line, the more personalized you get." In the future, personalization might take the form of "a melanoma chip that looks at certain single-nucleotide polymorphisms from your blood DNA and certain markers on the tumor, combined into some model that allows you to make a treatment decision." The model will consider germline variations as well as somatic mutations. "For instance, we know that if you inherit certain variants in thiopurine methyltransferase (TPMT) or HLA subtypes, you're going to be predisposed to toxicity in certain drugs, and

that information too will become more important as we get better and better drugs."

MOLECULAR DIAGNOSTIC TESTING COMES OF AGE

A variety of molecular technologies are being increasingly utilized in clinical practice, including polymerase chain reaction (PCR), comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), DNA/oligonucleotide microarrays, and DNA/RNA sequencing. But for the most part, the day-to-day performance of molecular testing in dermatology has lagged somewhat behind that of other specialties, particularly oncology, infectious diseases and clinical genetics, said a dermatopathologist who edited a textbook on the topic (*Molecular Diagnostics in Dermatology and Dermatopathology*, Humana Press/Springer, 2011). "Our specialty has been a little slow in adopting molecular testing. In many ways, we're victims of our own success," remarked Michael J. Murphy, MD, associate professor of clinical dermatology at the University of Connecticut School of Medicine and attending dermatopathologist at the University of Connecticut Health Center. "Amongst other contributing factors, the skin is readily accessible, so diseases are detected at early stages or repeat biopsies can be easily performed in equivocal cases. We have become very good at correlating histopathological findings with clinical information. Thus, there has been less impetus for us to embrace new technologies quickly."

Molecular diagnostics in dermatology will continue to evolve as a result of rapid technological advances.

While some dermatologists might question the immediate clinical relevance of molecular diagnostics, it's incumbent on them to stay abreast of these technologies and their developing applications, and incorporate such testing into diagnostic, prognostic, and therapeutic algorithms of patient care when appropriate, Dr. Murphy maintained. "If we don't become more educated and involved, we risk relinquishing the management of patients with certain skin diseases to other medical specialties. If future treatments are based not only on the clinical-histopathological features of a disease but also on its molecular changes, other physicians who are more informed may assume greater roles in patient care. We need to move to the next stage." Toward that end, many national and international dermatology and dermatopathology conferences are offering lectures and symposia dedicated to these advances, he remarked. "Recognizing the need and opportunity to integrate this growing discipline into a structured approach, we lecture on this subject as part of the dermatology residency curriculum here at UConn," he added. "This ensures that our residents are familiar with the applications of cutting-edge laboratory-based and dermatology-focused tests as part of their training."

In an article published in the *International Journal of Dermatology* (2012;51:1292-1302), Dr. Murphy and co-authors Zendee Elaba, MD, and Amanda Phelps, BA, explored the potential uses of molecular diagnostics in dermatology. In addition to testing for BRAF gene mutations in melanoma, they noted that recent studies have demonstrated a role for CGH and FISH in the evaluation of melanocytic tumors that are difficult to classify by conventional light microscopy. They also cited studies suggesting that CGH, FISH, and DNA microarray technologies "may potentially be used to stratify patients into prognostically relevant groups and provide biomarkers of treatment response and/or survival in patients with melanoma." Molecular technologies will also begin to play a greater role in the "diagnosis and management of other non-melanoma skin cancers, inflammatory dermatoses, and inherited skin disorders, in addition to dermatologic infections" the authors said, adding that nucleic acid-based testing could efficiently characterize "microorganisms that are difficult to culture, and uncover genetic determinants of disease outcome and/or treatment response, such as drug resistance genes."

Inflammatory dermatoses pose a particular challenge, Dr. Murphy said. "These are complex, often multifactorial and chronic, immune-mediated disorders with both polygenetic and environmental influences." However, he suggested that "atopic dermatitis and psoriasis are two conditions where the concept of 'personalized medicine' (i.e., tailored therapy) is likely to be realized first." An article discussing how genetic variation affects psoriasis patients' response to therapy, co-authored by two British researchers and published in *Expert Review of Clinical Immunology* (2010;6(6):957-966), noted that to date, there has been limited pharmacogenetic research regarding treatments for psoriasis. Based on the studies they reviewed, the authors concluded that developing targeted therapies for psoriasis

would involve "the combination of several genetic markers (polymorphisms) identified by large-scale gene association studies, each with a small but significant effect on treatment response, used in combination with additional clinical parameters, to reliably prospectively predict drug response." (One potential psoriasis target, REG3A, has since been identified; see www.aad.org/dermatology-world/acta-eruditorum/2012/september.)

Molecular diagnostic testing should not be employed in isolation, Dr. Murphy emphasized, and both the choice of test and significance of results must be determined in the context of available clinical and histopathological findings. This approach promotes even greater cooperation between dermatologists and dermatopathologists (and pathologists), for economic as well as clinical reasons, he said. "Closer ties among clinical and laboratory-based physicians will be necessary to ensure proper test platform selection with an understanding of test advantages and limitations, appropriate specimen handling, and accurate assessment of test results." As more molecular diagnostic assays become available, dermatologists will need to consider if a particular test is both clinically useful and cost-effective, Dr. Murphy added. "Broader acceptance of molecular diagnostics in dermatology will be linked to the timely acquisition of sensitive and specific actionable results which improve patient care and outcome." He noted that in studies from the dermatology literature which focus on comparative cost analyses in specific conditions, researchers found that in the setting of suspected dermatophytoses and cutaneous T-cell lymphomas, the costs of PCR-based diagnostic tests are similar to those of standard investigations, such as tissue culture and immunohistochemistry.

In the future, the most significant developments in molecular diagnostic testing will come through the use of next-generation sequencing, Dr. Murphy predicted. "Molecular diagnostics in dermatology will continue to evolve as a result of rapid technological advances and the acquisition of more affordable whole-genome data," he added. "Every few years, we can expect the discovery of novel disease-related genes for many skin disorders, the development of more reliable, robust, and accurate but less expensive molecular assays, and the introduction of more targeted, less toxic, single or combination therapies." ***dw***