Melanoma Assays in Development

Cancer of the skin is by far the most common cancer and is typically non-lethal. However, melanoma, while accounting for only about 2% of skin cancers, is responsible for the majority of skin cancer deaths. The American Cancer Society estimates that over 75,000 new cases of melanoma will be diagnosed in 2014, and that nearly 10,000 people will die of melanoma this year. While new and promising treatment approaches continue to emerge, melanoma is a diverse disease, and improved diagnostics for classifying the disease are required to use these new treatments most effectively.

The predominant mutation associated with melanoma, reported in approximately 50% of cases, occurs at position 600 in BRAF, a protein that is part of the mitogen activated protein (MAP) kinase signal transduction pathway. Approximately 80-90% of these BRAF mutations are known as V600E, a T>A switch that results in an amino acid change of valine to glutamic acid at position 600, within the activation portion of the BRAF kinase domain. Another 5-10% of mutations at the same position are known as V600K, which results in a lysine substituted for valine at the same position.2,3

Around 15-25% of melanomas display mutations in NRAS, a signaling molecule upstream of BRAF in the MAPK pathway4, while approximately 15% of melanomas show a mutation in KIT, a cell surface receptor involved in the same signaling pathway.5

Targeting tumor mutations
It is important to determine tumor genotype to choose the most effective therapy for the patient. For example, BRAF inhibitors have proven to be effective, at least initially, in extending progression free and overall survival6. Keith Flaherty, MD, director of Developmental Therapeutics at the Massachusetts General Hospital Cancer Center, Boston, was lead author of the 2010 New England Journal of Medicine article that announced results from phase 1 of clinical trials for the first approved BRAF inhibitor, vemurafenib.7

As Flaherty commented at the time8, "Until now, we've never had a credible first treatment option for metastatic melanoma, so this has completely transformed how we approach treatment for patients with the BRAF mutation." However, use of BRAF inhibitors in tumors with wild-type BRAF can have the opposite of the desired effect, leading to increased proliferation, highlighting the importance of accurate genotyping before using these agents.9

It is also useful to monitor tumor genotype during treatment. Tumors can be quite resourceful when subjected to selectional pressure from inhibitors targeting specific signaling pathway components. Tumor cells will often develop secondary mutations that activate alternate pathways to circumvent targeted therapies and allow the tumor to again grow. Typically, BRAF itself does not mutate additionally in response to inhibitor treatment, rather other upstream or downstream elements in MAPK pathway mutate, such as NRAS10 or MEK.11

The melanoma field has long awaited additional advanced diagnostic tools for characterizing tumors to facilitate a personalized medicine approach. Tools have emerged, with more on the horizon, although some may not be immediately available for clinical use.

Mutation detection – BRAF V600E
The first US Food and Drug Administration (FDA)-approved clinical diagnostic assay for melanoma genotyping, and the current standard, is the Roche cobas® 4800 BRAF V600 Mutation test. The Roche
test is a real-time PCR assay that can detect the V600E mutation in FFPE (formalin-fixed, paraffin-embedded) melanoma tissue samples. The assay has demonstrated >99% sensitivity in detection of the BRAF V600E mutation when compared with Sanger sequencing, with a lower limit of detection of mutant alleles <4%-5%.12

In 2013, a UK panel of melanoma experts recommended that all high-risk melanoma patients, ie, those with Stage IIb and higher, receive the V600E test.13 Despite the utility of the Roche BRAF Mutation Test for V660E, however, 50% of melanoma patients are wild-type for BRAF and will need additional testing.

Melanoma panel including NRAS
Until recently, no test was available for the 15-25% of melanoma patients with mutations in NRAS. However, a California company, DiaCarta, recently received CE/IVD status for a melanoma mutation detection panel that includes not only BRAF at position 600, but also NRAS, detecting mutations at codons 12 and 13 (exon 2), codons 59 and 61 (exon 3), and codons 117 and 146 (exon 4) of the NRAS proto-oncogene.14 The assay, based on real-time PCR, also tests for a mutation of cKIT, a protein that acts upstream of NRAS in the MAPK pathway, at codon 816 (exon 17) in the c-KIT proto-oncogene.

According to DiaCarta’s Chief Scientific Officer, Dr. Michael Powell, the assay is both fast and sensitive. “Our assays take less than 2.5 hours from receipt of sample to result and are capable of detecting as little as 0.1% mutant DNA from tumor biopsies or FFPE samples.” When asked to comment about the source of the speed advantage, Powell said, “Our sample prep method is carried out in a single tube, the contents of which are then used directly in our assays. This feature not only gets results sooner, but also greatly reduces error rates in processing DNA from clinical samples. The worst thing about such errors is that they provide mainly false negative results.”

Informed immunotherapy
Ipilimumab, a monoclonal antibody to CTLA-4 (cytotoxic T-lymphocyte antigen 4), stimulates the immune system and is approved for use in unresectable or metastatic melanoma.15 Some of the most promising current therapies for melanoma involve combining targeted inhibitors such as vemurafenib with immunotherapy agents.16

With the success of immunotherapies for the treatment of melanoma, interest in immunological biomarkers has grown as tumor classification has been seen to be important for immunotherapy treatment. In a recent study, patients with immunity to the antigen, NY-ESO-1, were seen to be 50% more likely to receive benefit from immunotherapy CTLA4 antibody ipilimumab.17

S6 phosphorylation as biomarker for inhibitor resistance
In terms of proteomic assays to characterize melanoma, a potential clinical diagnostic assay involves detection of the phosphorylation state of ribosomal protein S6. In xenograft studies, a decrease in P-S6 (detected by quantitative immunofluorescence microscopy) correlated with sensitivity to combined treatment with the BRAF inhibitor vemurafenib and the MEK inhibitor selumetinib. Tumors that did not display a decrease in P-S6 protein could be predicted to be resistant to the combined therapy.18

Although measuring a change in P-S6 after initiating treatment would require a second tumor biopsy from the patient, melanoma cells for the technique can be collected by fine needle aspiration, which is less invasive and safer than surgically-obtained biopsies. For an individual patient, such an indication of dual resistance would not yield a positive prognosis; the authors commented that, “if the information obtained through this approach can more effectively predict who is most or least likely to benefit from
therapy, the ability to spare patients the added cost and potential toxicity of an ineffective therapy and the opportunity to switch to a potentially more active agent or combination of agents may justify the additional procedure.”

**Future possibilities**
Microarrays continue to hold promise as tools that can take a genome-wide view to support personalized medicine in the treatment of malignant melanoma, but remain at the experimental stage. Research in the emerging field of epigenetics has offered tantalizing glimpses of the potential of detecting changes DNA methylation state or chromatin remodeling, not only across the entire genome, but particularly in association with genes known to be involved in the progression of melanoma. Non-coding RNAs of various lengths may also eventually have direct clinical relevance.19

**References**


14. QClamp™ Melanoma Mutation Panel, BRAF, NRAS and c-KIT. DiaCarta, Inc., Hayward, CA.


