What does *BRAF* testing tell us about melanoma?

*BRAF* is a gene that encodes for the serine/threonine-protein kinase *BRAF*. *BRAF* is part of the mitogen-activated protein kinase (MAPK) pathway. About 50% of melanomas carry the mutated form of *BRAF*. *BRAF*-mutated forms of melanoma tend to be more aggressive than forms of melanoma that do not contain the *BRAF* mutation (wild-type tumors). Targeted therapy with BRAF/MEK inhibitors can be used to treat *BRAF*-mutated melanomas.

Is *BRAF* the only mutation that can be targeted specifically in melanoma?

No. Other genetic mutations can be targeted. MEK inhibitors, which are frequently used in combination with BRAF-targeted therapies, work by targeting the signaling MAPK pathway downstream of *BRAF*. Now, with targeted exome sequencing, we can more easily recognize other therapeutic targets in melanoma. For example, a small proportion of melanomas carry mutations in c-*KIT*. Though rare, c-*KIT* mutations are present in some acral melanomas and mucosal melanomas (such as occurring on the anorectal region, vagina, or mouth). They can also occur in cutaneous melanomas, particularly in areas of chronic sun damage. Imatinib or nilotinib can be used to target c-*KIT* mutations. Similarly, a small proportion (<1%) of patients with melanoma harbor a *neurotrophic tropomyosin receptor tyrosine kinase (NTRK)* gene fusion. For such patients, an FDA-approved NTRK inhibitor, such as larotrectinib, would be appropriate.

Further Reading:

When should the \textit{BRAF} test be ordered?

The \textit{BRAF} test should be ordered at the time of diagnosis of Stage III or Stage IV disease. Some patients with Stage II disease might be tested as part of a clinical trial.

What type of tissue is required?

A biopsy is required to confirm metastatic disease. Therefore, it’s possible to use freshly acquired tissue or archival tissue for patients with metastatic disease. If the primary tissue is being considered for testing, it’s reasonable to use that sample if the tumor is thick enough to include the clones that were ultimately metastatic. However, if the patient had multiple prior melanomas, it might be best to wait for biopsy results from the metastasis to test. In general, if the patient has a primary melanoma with adequate tissue and a \textit{BRAF} mutation is found, we can trust that the \textit{BRAF} mutation will be present in subsequent metastatic disease.

Which specific \textit{BRAF} mutation do we need to test for?

\textit{BRAF}-targeted therapy is approved for patients with the \textit{V600E} and \textit{V600K} mutations because those are the dominant \textit{V600} mutations, and there are substantial data on randomized trials to suggest that these drugs are beneficial. Some of the \textit{BRAF} tests are specific for \textit{V600E} mutations, which are the more common of the 2. However, depending on where you are in the world, an estimated 5\%-30\% of \textit{V600} mutations can be \textit{V600K} and a few percentage can be others, \textit{V600R} or \textit{V600D}.

The percentage of \textit{V600K}, which is more closely associated with chronic sun damage, is higher as you go closer to the equator. MD Anderson, in its series in Houston, has higher rates of \textit{V600K} mutations than you would see in Boston, for example. Similarly, Australia has a very high rate of these \textit{V600K} mutations because of the chronic sun damage and the UV radiation that’s probably driving that type of mutation. Now, in this population there are some case reports suggesting that patients with other \textit{V600} mutations, such as \textit{V600R} or \textit{D}, may also benefit from \textit{BRAF}-targeted therapy. There are no randomized data in those populations.

Ideally, you need a molecular assay that can detect either the \textit{V600E} or the \textit{V600K} mutation.

Further Reading:


Further Reading:


What types of BRAF testing are available? How do they compare?

The range of options, shown in the table, includes companion diagnostics, immunohistochemistry (IHC) staining, next generation sequencing, and a blood test. The cobas® 4800 BRAF 600 Mutation Test (Roche Diagnostics) test, which is a PCR-based companion diagnostic test, is very good at identifying V600E. However, it’s not particularly good at identifying V600K. The THxID®-BRAF kit (bioMérieux), which is also a companion diagnostic, is very good at identifying both V600E and V600K.

Another is the BRAF V600E stain test. An IHC stain test, it takes 24 to 48 hours to yield results, and it is only specific for V600E, which is the majority of V600 mutations. However, the V600E mutation rate can be low, depending on the geographic location. In addition, you may also discover a V600K mutation when the test results come back negative for V600E. So, a negative IHC stain test does not mean the V600K mutation is not present.

Targeted exome sequencing enables one to look at 60, 100, or even 200 genes. You’re looking for a hot spot of mutations as well as any alterations on mutations, changes in copy numbers, or even fusions. This could include patients with NTRK fusions. Finally, there are now standard blood assays. Those tend to be next generation sequencing approaches to identify those mutations.

Table: Options for BRAF Testing

<table>
<thead>
<tr>
<th>Name of Test</th>
<th>Test Method</th>
<th>Turnaround Time</th>
<th>Mutations Tested for</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THxID™ BRAF (bioMérieux, Inc.)</td>
<td>Real-time PCR</td>
<td>&lt;1 week</td>
<td>V600E and V600K</td>
<td>&gt;96 for V600E</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>92 for V600K</td>
<td></td>
</tr>
<tr>
<td>cobas® 4800 BRAF V600 (Roche Diagnostics)</td>
<td>Real-time PCR</td>
<td>&lt;1 week</td>
<td>V600E and V600K</td>
<td>~97 for V600E</td>
<td>&gt;98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>66-70 for V600K</td>
<td></td>
</tr>
<tr>
<td>Immunohistochemistry (IHC) (Ventana Medical System)</td>
<td>VE1 antibody against the BRAF V600E mutated protein</td>
<td>24-48 hours</td>
<td>V600E</td>
<td>89.2</td>
<td>96.2</td>
</tr>
<tr>
<td>FoundationOne CDx (Foundation Medicine, Inc.)</td>
<td>Next Generation Sequencing, multiple instruments</td>
<td>&lt;2 weeks</td>
<td>V600E, V600K, BRAF V600 dinucleotide</td>
<td>99.4, 99.3, and 96.3 positive percent agreement, respectively</td>
<td>89.6, 99.2, 100 negative percent agreement</td>
</tr>
<tr>
<td>Blood assay (next generation sequencing) (Foundation Medicine, Guardant Health; Guardant 360)</td>
<td>Next generation sequencing for circulating free tumor DNA</td>
<td>&lt;9 days</td>
<td>V600E and other mutations</td>
<td>38–79</td>
<td>40–100</td>
</tr>
</tbody>
</table>
Further Reading:


How can you fast track a test?

You might select your test based on how quickly you need the results. If you need results in 24 hours, do the immunohistochemical stain. If you need results within a week, do the companion diagnostic or a test similar to that. If you want as much information as possible to understand whether or not there's a mutation that's actionable, you should do an expanded panel that includes fusion proteins, that includes *BRAF* and *NRAS* and *KIT*—and dozens of other genes that can really provide a more comprehensive understanding of what's going on with that. There’s no reason not to order the immunohistochemical test plus another test to gain additional information.

What should be done when there is not enough tissue?

Multiple types of sample(s) can be used including formalin-fixed, paraffin-embedded tissue, unstained slides, or cytology smears, if there is enough tissue. Now that there is a blood assay, that provides another option. If all else fails, you may opt to obtain an additional biopsy.
What kind of general education do you provide?

It’s important to educate patients about what **BRAF** is. We tell patients that **BRAF** is a gene that tells your cells how to grow. A **BRAF** mutation is a change in a **BRAF** gene. That change in the gene can lead to an alteration in a protein that regulates cell growth that could allow the melanoma to grow more aggressively. Approximately half of melanomas carry this mutation and are referred to as mutated, or **BRAF**-positive, melanomas. Those that do not carry the mutation are referred to as wild-type, or **BRAF**-negative, melanomas.

Many patients are confused about whether the **BRAF** mutation is inherited. It’s important to help them understand that it is not found in the sperm/eggs—it’s a mutation that occurs in the tumor itself. So they can’t inherit it or pass it on to their children.

How do you advise patients who have a positive **BRAF** test?

We advise them that there is a therapy specific to the **BRAF** mutation. It is not chemotherapy: it’s designed to target their specific type of tumor. Traditional chemotherapy does not work very well in melanoma. Targeted therapy, on the other hand, is more “specific” and has much better outcomes than those seen with chemotherapy. Targeted therapy has side effects that differ from those of chemotherapy. You won’t see the classic side effects such as hair loss, nausea, and vomiting that are associated with chemotherapy. Targeted therapy has a different set of side effects, which can include fever, skin reactions, and cardiac and ocular side effects.

Some patients worry that if they are **BRAF** positive, then they will have to take the **BRAF** “drug” before they’re allowed to “take the effective medicine—immunotherapy.” That’s not true. First, both targeted therapy and immunotherapy are really good medicines. Second, since patients with **BRAF** mutations are eligible for either targeted therapy or immunotherapy, they and their team will decide what’s best in terms of which type of agent will be used to start Stage IV treatment. There is no set order for how these drugs are to be given. In the Stage III setting, after surgery to remove the melanoma, the patients will work with their provider to determine which therapy is best for them.

How do you advise patients who have a negative **BRAF** test?

We tell them they most likely will be eligible for immunotherapy and potentially therapies being studied in clinical trials.