ADVANCES IN DRUG DEVELOPMENT

Current Developments in Oncology Drug Research

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BRAF Validation in Melanoma

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H&O What is BRAF and what role does it play in melanoma?

KF V-raf murine sarcoma viral oncogene homolog B1 (BRAF) is one of the enzymes that are part of the MAP kinase pathway, which is arguably the best-studied signal transduction pathway in cancer. The MAP kinase pathway is one of several pathways that lie downstream of receptor tyrosine kinases or surface receptors that are the recipients of growth factor signals. These surface receptors, in the absence of cancer or mutations in this pathway, are turned on by the presence of growth factor ligands and consequently trigger signal transduction cascades inside the cytoplasm of the cell. Many of those signals are ultimately transmitted to the nucleus, which results in the alteration of gene expression or of the program or behavior of a cell.

BRAF mutations were first described in 2002. At present, approximately 7% of cancers harbor this mutation. We have known for the past 20 years that in cancer, there are mutations in rat sarcoma viral oncogene homolog (RAS), which is one of the immediate downstream components of the surface receptors and is another constituent of the MAP kinase pathway in addition to BRAF. RAS is capable of turning on several downstream pathways other than just BRAF, and the downstream components of the MAP kinase pathway: the PI3 kinase pathway, the RAL-GDS pathway, and 3 others, totaling 6 "RAS effector pathways." Under normal physiologic conditions, all these pathways are downstream of those receptors and RAS itself, which has 3 isoforms that are highly related. RAS mutations (in 1 of the 3 isoforms) are found in approximately 20% of all cancers.

One of the great challenges in oncology and therapeutics has been the fact that to this day, there are no effective RAS-targeted therapies. It has been a major frustration because RAS is a type of enzyme for which it is difficult to develop very specific enzymatic inhibitors. Thus, with RAS being an elusive target, there has been a great deal of interest in finding out whether there might be other elements of the MAP kinase pathway and other RAS effector pathways that might be mutated in cancer. Like RAS mutations, BRAF, when mutated, can drive signaling through the pathway downstream of itself independent of any upstream activation. So, just like RAS mutations, it is no longer important whether the growth factor receptors are turned on or not. There is some evidence that suggests that BRAF mutations only turn on the MAP kinase pathway and not other pathways, whereas RAS mutations turn on more than 1 pathway.

It is also known that another of the RAS effector pathways, the PI3 kinase pathway, commonly harbors mutations in approximately 25% of all cancers, and is therefore a quite common hotspot in terms of mutations. One can find cancers where there are both BRAF and PI3 kinase mutations, but generally if a RAS mutation is present, there will be no BRAF mutation.

H&O In which cancers do we see evidence of BRAF mutations?

KF Melanoma is the predominant home of BRAF mutations; approximately 50–60% of all melanomas will have mutations in BRAF. These mutations are also seen in 30–40% of papillary thyroid cancers, approximately 20% of cholangeocarcinomas, 10% of colorectal cancers (RAS mutations occur in 40–50% of colorectal cancers; this is a separate set of colorectal cancer patients who have BRAF mutations), and 10% of ovarian cancers. Smaller percentages of other cancers, including lung, breast, and testicular cancer, also harbor BRAF mutations. Most of the laboratory research has been done in melanoma and thyroid cancer, and in both cases it appears that BRAF is a good target based on in vitro and animal model work. What we still do not know but hope to sort out is the relevance of BRAF as a target in all the other cancers.

H&O Why was the discovery of BRAF mutations significant?

KF The discovery of mutations in BRAF in 7% of all cancers was an exciting development for 2 reasons. One is that BRAF and RAS mutations are mutually exclusive events that reinforce the biologic significance of each other; in other words, they do not exist in the same cancer cell. It is known that cancer cells harbor many mutations, and it takes numerous mutations to allow a cancer to form in the first place. The question is how many of these mutations are just genetic alterations that are innocent bystanders versus how many are critically important to cancer formation, metastasis, and ultimately death from cancer. RAS and BRAF are both thought to be the critical driver mutations—critical components instead of stand alone components in terms of cancer formation and misbehavior. So again, with RAS mutations being in a separate set of patients and their tumors, BRAF mutations were a new discovery and a distinct point in signal transduction pathways. What was also exciting about the discovery of BRAF mutations is that RAF kinases, including BRAF, are key kinases or enzymes that can be blocked with drugs. With this finding, BRAF became the most prevalent oncogene for which a drug could be directed. Even in 2002, when the discovery was first made, it was highly anticipated that it would be possible to develop potent and specific inhibitors to BRAF, which would then give us a tractable target that RAS never turned out to be.

H&O What was the landscape of melanoma therapy prior to the discovery of BRAF inhibitors?

KF When BRAF mutations were discovered, there was a single drug that was already in clinical testing: sorafenib (Nexavar, Bayer). Sorafenib inhibits many enzymes within cells, including RAF kinases. Although BRAF, CRAF, and ARAF were on the list of targets, they were not the main targets of this drug. At that time, my group at the University of Pennsylvania and other groups around the world were keenly interested in studying sorafenib to determine whether it might be a useful melanoma therapy. My group conducted several single-agent phase II trials of sorafenib in melanoma as well as several trials combining sorafenib with chemotherapy. Over the course of these studies, we found that sorafenib did not have much single-agent efficacy in patients with BRAF mutant melanoma. What was unclear from those studies was whether the lack of efficacy was because BRAF itself was not a critical target or because sorafenib was not effective in blocking BRAF. Both of these possibilities were examined in the several years during which we conducted trials with sorafenib. Eventually, 3 randomized trials evaluating chemotherapy with or without sorafenib were conducted. The study findings showed that sorafenib did not add anything to the effectiveness of chemotherapy in melanoma patients.

H&O What agents are currently under investigation?

KF In late 2005, the first 2 professional BRAF inhibitors were entering phase I trials, and soon after there were 2 more that were entering trials. The BRAF inhibitors that are currently in clinical development can be grouped into 2 categories: drugs that are very selective BRAF inhibitors and drugs similar to sorafenib in that they block/inhibit multiple kinases but have particular potency against BRAF. At this time, we do not know which group has superior efficacy. Another group of drugs that are being studied in BRAF mutant melanoma are MEK inhibitors.

Selective BRAF Inhibitors

One drug that is coming out of phase I/II trials is a very selective BRAF inhibitor, PLX4032 (Plexxikon). Results of the phase I part of testing were presented at the 2009 American Society of Clinical Oncology (ASCO) meeting, when patients were being recruited into the phase II portion of the study. To date, PLX4032 has been tested in the largest group of melanoma patients compared to any of the other drugs. Forty-nine of the 55 patients who went on to the phase I portion of the study had metastatic melanoma. Before being enrolled in the trial, the vast majority of patients were prescreened at each of the 6 centers to determine whether their tumors had BRAF mutations. At the end of the phase I portion of the study, a phase II extension study was conducted. In the extension study, an additional 32 patients with metastatic melanoma and BRAF mutations were treated at the phase II dose (960 mg twice daily). The safety analysis revealed several toxicities that were related to the dose of the drug being administered. Side effects—all of which were mild to moderate in severity, reversible, and manageable—were seen with some frequency at higher doses. Typical toxicities were skin toxicities: rash and sun sensitivity. Fatigue, arthralgia, and benign skin lesions (keratoacanthoma) were also reported. When upper levels of dose escalation were reached in the phase I portion of the study, we began to see responses by conventional CAT scans using Response Evaluation Criteria In Solid Tumors (RECIST). Sixteen patients with metastatic melanoma who had BRAF mutations and were enrolled across the top 5 dose levels, and 5 additional metastatic melanoma patients who did not have BRAF mutations at the same dose levels, were available for evaluation. Of the 16 patients, 9 had objective responses by RECIST

criteria, whereas none of the patients without BRAF mutations responded. Updated during the joint Congress of the European CanCer Organisation and Congress of the European Society for Medical Oncology (ECCO/ESMO) in September, 11 of the same 16 BRAF mutated melanoma patients had achieved a partial response (69%). The level of activity among this small cohort of 16 patients is a sign that PLX4032 has single-agent activity and that BRAF is an important target in melanoma.

Results of the phase II portion of the study were presented at the ECCO/ESMO meeting. Among the additional 32 patients enrolled in the extension study, there was a response rate of 70% by RECIST criteria; this rate matched the response seen in the phase I portion of the study. In melanoma, the typical response rates seen with dacarbazine or high-dose interleukin-2 are 10–15%, so compared to this reference, the 70% response rate observed in our studies was of great interest. There are now 2 ongoing trials that are seeking to produce data sufficient to get approval from the U.S. Food and Drug Administration (FDA) and global regulatory bodies. One study is a phase II trial of PLX4032 as a single agent in patients who have already failed standard therapy for metastatic disease (dacarbazine or interleukin-2). This is an uncontrolled, single-arm phase II trial that will hopefully confirm the high response rate we have seen with this drug. The other study is a phase III trial comparing single-agent PLX4032 to single-agent dacarbazine. This study is a head-to-head comparison, which plans to enroll approximately 700 patients who have not received previous therapy for metastatic disease. The primary endpoint will be overall survival.

Another selective BRAF inhibitor is the GlaxoSmithKline drug GSK2118436. This drug is also in phase I testing; results are expected some time in the spring or summer of 2010.

Nonselective BRAF Inhibitors

XL281 (Bristol-Myers Squibb) is a relatively nonselective inhibitor. It is the only other BRAF inhibitor for which we have any clinical data. It is at the end of phase I testing and in phase II evaluation. Phase I results were presented at the 2009 ASCO meeting. Another less selective BRAF inhibitor, RAF265 (Novartis), is currently in phase I testing. No results have been presented for this drug as of yet.

MEK Inhibitors

There was a period of time when the field focused heavily on the possibility of blocking MEK in BRAF mutated cancers, the enzyme that is immediately downstream of BRAF. MEK is a kinase and is part of the MAP kinase pathway. It is never mutated in cancer, but is activated by BRAF and in turn activates the rest of the MAP kinase pathway. In the laboratory it is evident that MEK inhibitors can be useful for BRAF mutant cancers.

Although we do not have great comparative literature, there is some evidence that BRAF inhibition is a strategy superior to MEK inhibition, specifically in BRAF mutant cancers. Currently, there are numerous MEK inhibitors in clinical trials. AZD6244 (AstraZeneca) is the MEK inhibitor that has been evaluated most extensively in melanoma, specifically in BRAF mutant melanoma. Results of a phase II study that compared AZD6244 to temozolomide (oral version of dacarbazine) found that of 45 BRAF mutant patients, objective response was seen in 5 patients (12%). The patients in the study who received temozolomide had a similar response rate and progression-free survival. Thus, we have an early hint that this drug, as a MEK inhibitor, is not a superior treatment to chemotherapy (temozolomide).

There are numerous other MEK inhibitors in clinical development, and it remains possible that there may be a better option than AZD6244. However, more research is necessary to determine whether MEK inhibition is comparable to BRAF inhibition.

H&O Do BRAF inhibitors show potential as part of combination therapy?

KF There have been active discussions in the field about combining PLX4032 with other targeted agents. Because there are other mutations that activate the PI3 kinase pathway in melanoma, we believe that combining a BRAF inhibitor like PLX4032 with potent and specific inhibitors of other key signaling molecules might produce more efficacy that PLX4032 alone.

Immunotherapies are also under investigation in melanoma; several very new, targeted immunologic therapies that are in late-stage clinical trials have shown the type of results that may lead to FDA approval. Combining PLX4032 or BRAF inhibitors like it with these immunotherapies is also of high priority in research.

H&O What is the widespread application of BRAF?

KF The hope with BRAF inhibitors is that they can be applied not just in melanoma patients, but in other cancers as well. We have found that testing for BRAF mutations upfront is something that is highly practical and beneficial for determining whether a specific therapy is effective in patients with this mutation. BRAF screening is something we have been doing routinely in trials for patients with melanoma and in the context of clinical trials in patients with colorectal cancer (BRAF mutations appear to confer resistance to EGFR targeted therapies,

as do KRAS mutations). In the next year, we anticipate learning the implications of BRAF as a target across all cancers in which it is found. Melanoma is in the lead in terms of the disease where this target is most prevalent, and it is going to continue to be the entity where we will gain the first insights into the clinical significance of BRAF targeted therapies. The hope is to gain a better understanding of the other diseases that harbor this mutation soon after.

Suggested Reading

Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature.* 2002;417:949-954.

Smalley KS. Understanding melanoma signaling networks as the basis for molecular targeted therapy. *J Invest Dermatol.* 2010;130:28-37.

Chapman P, Puzanov I, Sosman J, et al. Early efficacy signal demonstrated in advanced melanoma in a phase I trial of the oncogenic BRAF-selective inhibitor PLX4032. *European Journal of Cancer Supplements.* 2009;7:5.

Flaherty K, Puzanov I, Sosman J, et al. Phase I study of PLX4032: proof of concept for V600E BRAF mutation as a therapeutic target in human cancer. *J Clin Oncol (ASCO Annual Meeting Abstracts).* 2009;27:Abstract 9000.