BRAF Inhibitor Unveils Its Potential against Advanced Melanoma

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Unresponsiveness to therapy is a hallmark feature of advanced metastatic melanoma. However, the discovery of BRAF-activating mutations in approximately 50% of human melanomas has provided an attractive therapeutic target. Here, we discuss two recent publications focusing on the mutant BRAF kinase inhibitor PLX4032 that validate oncogene-targeted melanoma therapy.

Traditional approaches to treat advanced metastatic melanoma benefit only a small subset of patients and provide response rates rarely above 20%. A new wave of therapeutic strategies may drastically change this dismal outlook as more specific pharmacologic inhibitors, well-defined molecular targets, and immunotherapies are significantly impacting disease outcome.

Melanomas are comprised of clinically and genetically distinct subgroups (Curtin et al., 2005), indicating the potential for individualized therapies. The discovery that melanomas harbor activating mutations in the serine-threonine BRAF kinase in approximately 50% of patients (the V600E mutation being the most common) (Davies et al., 2002) prompted an intense search for compounds to inhibit BRAF activity. The first clinical drug candidate, sorafenib, provided disappointing results even when combined with chemotherapy (Flaherty et al., 2008; Hauschild et al., 2009). Sorafenib is a broad-spectrum kinase inhibitor with higher potency against CRAF than BRAF; therefore, searching for more selective inhibitors was necessary to validate the effectiveness of targeting mutant BRAF in melanoma.

As recently reported in *Nature,* Bollag et al. (2010) used a structure-guided approach to develop a kinase inhibitor, PLX4032 (also known as RG7204), that is selective for BRAF V600E. PLX4032 is a well tolerated, orally available small molecule inhibitor with remarkable selectivity against BRAF mutant cells in vitro and in mouse xenograft models (Bollag et al., 2010; Lee et al., 2010). Despite encouraging preclinical studies, no tumor regression was observed in the initial phase 1 clinical trial with PLX4032, even though phosphorylated ERK (pERK) levels were reduced in tumor biopsies. PLX4032 was then reformulated in a collaboration between Plexxikon and Roche to allow higher bioavailability. Reformulated PLX4032 achieved greater than 80% inhibition of pERK levels and strong tumor response (Bollag et al., 2010).

In *The New England Journal of Medicine*, Flaherty et al. (2010) provide the breakthrough clinical evidence showing that PLX4032 treatment of metastatic melanomas harboring the $BRaf^{V600E}$ mutation results in complete or partial tumor regression in the majority of patients. In an extension phase of the study using the maximum tolerated dose, 81% of patients (26 of 32) had tumor regression according to the Response Evaluation Criteria in Solid Tumors (RECIST) (Flaherty et al., 2010). Responses are not observed in patients with tumors carrying wild-type BRAF, indicating that oncogene-targeted therapy via mutant BRAF inhibition is a specific and valid strategy exclusively for the treatment of BRAF mutant tumors.

These studies not only establish mutant BRAF as a bona fide therapeutic target but also position PLX4032 as the first selective BRAF inhibitor to display clinical activity in BRAF mutant melanomas. Although debates arise on the issue of selectivity of anticancer drugs and their ability to kill tumor cells given the complex signaling networks involved, PLX4032 data indicate that being "on-target" has its advantages. First, the high blood levels needed to obtain a clinical response may not have been achieved with a less selective and thus potentially more toxic compound. Second, the clinical response can be obscured when too many "target hits" are involved. This may not be a disadvantage if positive clinical results are obtained, but if therapies are unsuccessful, knowing where and how to troubleshoot is key. Finally, with the multiple agents available for combinations, knowledge of a drug's mode of action will facilitate compound selection and patient assignment for proper individualized therapy. So far, analysis of tumor biopsies indicate that PLX4032 "hits" its intended target, assessed via decreases in intratumoral levels of pERK, which best correlate with response, and near-complete inhibition of ERK signaling seems to be necessary to cause significant tumor regression (Bollag et al., 2010).

Encouragingly, side effects of PLX4032 are manageable and consist of grade 2–3 rashes, fatigue, joint pain, and cutaneous squamous-cell carcinomas (SCC), keratoacanthoma-type lesions. These cutaneous lesions appeared in 31% of patients; however, they are well differentiated and have low invasive potential (Flaherty et al., 2010). They appeared in sun-exposed skin areas and were also observed following sorafenib, XL281, and GSK2118436 treatments, suggesting that pre-existing oncogenic mutations may potentiate RAF inhibitor-mediated side effects. Interestingly, PLX4032 appeared to have no effects on benign nevus progression or regression (Bollag et al., 2010); the senescence state of these lesions could account for this lack of response.

So far, the biggest concern regarding PLX4032 and likely all BRAF inhibitors arises from the fact that responsive tumors eventually acquire resistance to

the compound. Currently, the median progression-free survival is estimated at more than 7 months; however, the median overall survival rate is not yet available. Why and how resistance is acquired is being intensively studied. Subpopulations of non-drug-responsive cells may be present in the tumor and slowly overtake the drug-sensitive populations, or possibly all tumor cells respond to the compound but not to the same extent, allowing some cells to survive while they rewire for growth. The only way to address this issue is to study the phenomenon in preclinical models that closely mimic what occurs in the clinic, and importantly, to have access to patient samples following clinical trials to validate the findings. Resistance (acquired and/or intrinsic) could involve multiple counterbalancing pathways, molecular culprits, or genetic alterations; however, so far Flaherty et al. report that ''gatekeeper'' BRAF mutations have not been detected (Flaherty et al., 2010).

The melanoma field is no longer facing an insurmountable treatment wall and multiple options are emerging now that mutant BRAF-based therapy shows positive outcomes. For example, trials could combine BRAF inhibitors with compounds targeting other melanoma-relevant signaling networks (such as the PI3K pathway) or combinations with immunotherapy (such as the recently clinically successful ipilimumab, which targets CTLA4) (Hodi et al., 2010). The emergence of other specific BRAF inhibitors such as GSK2118436 may soon confirm the robustness of targeting mutant BRAF while expanding the arsenal of antimelanoma inhibitors available to patients. It is hoped that each clinical trial is preceded by solid preclinical data as multiple models are available to systematically assess the validity of combinations. For example, a new mouse genetic model of $BRaf^{V600E}/Pten^{-/-}$ mimics melanoma progression (Dankort et al., 2009). In addition, multiple human xenograft models exist that reflect the diversity of melanoma subgroups.

Positive results from the ongoing trials could sway the FDA toward an early approval despite the eventual recurrence of tumors. Regardless of the encouraging results, the melanoma field remains challenged with several issues such as (1) acquired resistance to PLX4032 and other BRAF inhibitors, (2) some BRAF mutant melanoma tumors that do not respond to BRAF inhibitors (intrinsic resistance), and (3) wild-type BRAF in 50% of melanomas. There are also many open questions regarding the signaling networks involved in melanoma: even if an ideal target is identified, how long and to what extent will compensatory mechanisms take over? Tumor heterogeneity would indicate that not all cells within one tumor are killed or respond equally (Roesch et al., 2010). Will this play a significant role in future treatment strategies? Will we need two therapies, one to eliminate the majority of the cells and another to target the minor subpopulation(s)?

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