Targeting BRAF in Advanced Melanoma: A First Step toward Manageable Disease

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Abstract

Melanoma is the deadliest form of skin cancer and its incidence has been increasing worldwide. The disease manifests itself as clinically and genetically distinct subgroups, indicating the need for patient-specific diagnostic and treatment tools. The discovery of activating mutations (V600E) in the BRAF kinase in approximately 50% of patients spurred the development of compounds to inhibit aberrant BRAF activity, and the first drug candidate to show promising clinical activity is PLX4032 (also known as RG7204). Most recent clinical data from a phase II trial indicate that PLX4032 causes tumor regression and stabilized disease in >50% of advanced melanoma patients harboring BRAF V600E tumors. These data validate the effectiveness of oncogene-targeted therapy against advanced melanoma and offer hope that the disease can be overcome. However, as melanoma is dynamic and heterogeneous, careful treatment strategies and combination therapies are warranted to obtain long-term clinical effects. Clin Cancer Res; 17(7); 1658–63. ©2011 AACR.

Background

Melanoma constitutes an important medical challenge, as it accounts for more than 80% of skin cancer deaths, and its incidence has increased over the past decades worldwide. This devastating disease arises from the transformation of melanocytes (specialized pigmented skin cells), in which accumulation of mutations in growth-regulating genes, increase of autocrine/paracrine growth factors, and loss of adhesion receptors cause uncontrolled proliferation, dissemination, and enhanced survival (1). As a result of many of these alterations, the mitogen-activated protein kinase (MAPK) pathway becomes activated in the majority of melanomas; thus, targeting this pathway holds great therapeutic potential.

Indeed, extensive data point to the key role of the classic MAPK pathway in modulating melanoma survival, proliferation, invasion, and angiogenesis. In normal cells, this pathway is turned on by the regulated expression of ligands, which bind to cell membrane-bound receptors. These receptors then recruit the small G-protein RAS, allowing its binding to the inner surface of the cell membrane to further activate the serine-threonine RAF protein kinase and also potentiate phosphoinositide 3-kinase (PI3K) signaling. The activated RAF kinase phosphorylates and activates the MAPK/extracellular signal-regulated kinase kinase (ERK1/2; MEK1/2), which further phosphorylates, activates, and allows the translocation of the ERK1/2 to the nucleus to regulate gene expression (2, 3). Figure 1 shows a simplified schematic of the classic MAPK pathway and its key effectors.

In melanoma, aberrant MAPK signaling is initiated by alterations in membrane receptors or mutations in downstream effectors such as RAS or RAF (4). NRAS mutations are found in about 20% of melanoma patients, but it is the RAS substrate BRAF that harbors the most frequent mutations in patients’ samples (50 to 70% of cases; ref. 5). Of these mutations, about 80% display a valine to glutamic acid substitution (V600E), causing constitutive kinase activation, and about 16% harbor a valine to lysine substitution (V600K; refs. 6, 7). MAPK signaling is required for proliferation of both RAS and RAF-transformed melanocytes, as it was shown that RAF and MEK inhibitors decreased ERK activity and blocked their cell cycle progression. Although mutant BRAF can act as a potent oncogene in the early stages of melanoma by signaling via MEK and ERK, it is not required in RAS-transformed melanocytes because of intrinsic pathway redundancy (8). Given the large number of melanomas that harbor activating mutations in the BRAF oncogene and their reliance on BRAF activity, targeted inhibition of this protein became of high interest.

Mutations in MEK are not frequent, and ERK mutations have not been identified in melanoma; however, as the MAPK pathway is persistently active in the tumor cells, these effectors can also be targeted. Results show that MEK inhibitors induce significant reduction in melanoma growth in preclinical models (9, 10). Nevertheless, MEK inhibitors have not shown significant clinical efficacy in melanoma clinical trials so far, and dose-limiting toxicities were observed (11). Interestingly, inhibitors of BRAF and

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MEK were reported to have similar transcriptional targets; therefore, MEK inhibitors could be useful in patients with acquired BRAF inhibitor resistance if toxicities can be managed (12).

Interestingly, BRAF V600E mutations are also observed in benign nevi; this suggests that BRAF mutations alone are insufficient for tumorigenesis and that additional factors are needed for cancer progression (13). In fact, a mouse genetic model of BRAF\(^{V600E}/PTEN^{−/−}\) that mimics melanoma progression indicates that the PI3K pathway also plays an important role in the development of aggressive tumors (14). Crosstalk between the MAPK and PI3K signaling pathways has been shown and can happen at multiple levels (15–17). In addition, PI3K pathway activity was shown to be increased in melanoma via constitutive activity in AKT3 (18) or through loss of activity of the tumor suppressor PTEN. This loss occurs through PTEN mutation, deletion, or methylation, which can also coincide with BRAF mutations but not NRAS (19). PTEN loss is found in 5 to 20% of noninherited melanomas, and similar to other neoplasia, may regulate inhibition of the MAPK pathway, cell cycle arrest, and survival via effects on Bcl-2 and caspas, for example (20). Thus, as melanomas favor the disregulation of both the MAPK and PI3K pathways, their combined targeting has therapeutic merit (Fig. 1; ref. 21). Although multiple other signaling pathways may be involved in melanoma oncogenesis, finding which ones are essential for melanoma survival and progression will determine their therapeutic value.

Clinical-Translational Advances

Despite the extensive scientific progress in the melanoma field, treatment of advanced stage melanoma with...
chemotherapeutics and biotherapeutics has rarely provided response rates higher than 20% (22). This dreary clinical picture is now changing as "personalized therapy" approaches and carefully designed small molecule inhibitors to treat metastatic melanoma are currently under study and being applied to patients with genetically characterized tumors (23).

The discovery that BRAF mutations are found in approximately 50% of melanoma cases (5) provided an opportunity to target a cancer-specific oncogene and develop compounds to curb its aberrant activity. The first clinical drug candidate aimed at inhibiting BRAF activity, sorafenib (Nexavar, Bayer), displayed disappointing results, even when combined with chemotherapeutics, and is believed to have failed because of its nonspecific broad-spectrum kinase inhibitor activity and associated toxicity (24, 25). However, newer, more potent, and especially more specific inhibitors, such as PLX4032 (also known as RG7204-Plexxikon/Roche), its related compound PLX4720, XL281 (Exelixis Inc.), and GS2118436 (GlaxoSmithKline), provide increasing proof that targeting BRAF in melanoma is a genuine therapeutic approach (Fig. 1; refs. 26–28).

The most clinically advanced of these BRAF inhibitors, PLX4032, was developed through structure-guided approaches and is a well-tolerated, orally available small molecule inhibitor with selectivity against BRAF mutant cells and tumors (29–31). Groundbreaking clinical results with this compound were unsealed through a phase I extension study of melanoma patients with BRAF V600E tumors, showing that PLX4032 treatment of these metastatic melanomas caused complete or partial tumor regression in 81% of patients [according to the Response Evaluation Criteria in Solid Tumors (RECIST); ref. 23]. Results from the phase II trial (totaling 132 patients) were recently presented by Dr. Jeffrey Sosman at the 7th International Melanoma Research Congress of the Society for Melanoma Research in Sydney, Australia, and indicate that 52% of patients show tumor size reductions of 30% or more, 82% of patients exhibit either a response (52%) or stable disease (30%), and that the median progression-free survival rate is 6.2 months (32, 33). New data also suggest that PLX4032 is effective against V600E/K tumors, and patients harboring these mutations should be included in future clinical trials (7). In sum, PLX4032 provides the first clinically convincing evidence that a personalized medicine, oncogene-targeted approach is valid for advanced melanoma.

One theory why BRAF inhibitors, such as PLX4032, are effective in BRAF mutant tumors and have a greater therapeutic index than MEK inhibitors is that they do not inhibit ERK activity in normal cells, thus decreasing toxicity (2). Another possible reason for the advantage of BRAF inhibitors over broad MAPK pathway blockade is that BRAF inhibition increases the presentation of melanoma antigens and subsequent recognition by T-lymphocytes, whereas MEK inhibition impairs T-lymphocyte activity (34). These observations must be taken into account for future combination therapies involving immunotherapies.

For example, treatment combinations with the recently clinically successful ipilimumab, which targets CTLA4, could potentially be beneficial to circumvent drug resistance (35).

Side effects still occur with PLX4032 but are generally manageable. PLX4032 phase I and II trials report grade 2–3 rashes, photosensitivity, hair loss, fatigue, joint pain, and well-differentiated cutaneous squamous cell carcinomas (in >30% of patients; refs. 23, 32). These lesions were also observed following sorafenib, XL281, and GS2118436 treatments and appeared on sun-exposed areas, indicating that RAF inhibition may exacerbate conditions associated with preexisting oncogenic mutations (29). The phase II study additionally reports the most severe side effects as being abnormal liver function (14% of patients), joint pain and/or arthritis (11% of patients), and gastrointestinal problems (10% of patients; ref. 32). Hopefully, as we gain a better understanding of PLX4032 and its effects in various patients, some of these side effects will become preventable.

Other mutant BRAF inhibitors are also gaining momentum clinically, such as GS2118436, and will also be closely monitored by the melanoma field. GS2118436 is a potent BRAF inhibitor with high selectivity for mutant BRAF compared with the wild-type protein. Similar to PLX4032, preclinical data indicate dose-dependent MEK and ERK activity inhibition and tumor regression in mouse models. In a phase I-II study, 60% (18 out of 30) melanoma patients with mutant BRAF had more than 20% tumor reductions by RECIST (28). Unprecedented positive results were also reported at the 35th Congress of the European Society for Medical Oncology (ESMO) by Dr. Georgina Long from Melanoma Institute Australia. These findings show that in a phase I-II GS2118436 trial, 9 out of 10 patients with untreated brain metastases had reductions in overall size of their brain lesions (36).

Although the ability to inhibit mutant BRAF to cause the regression of metastatic lesions is considered one of the most significant developments in the history of melanoma treatment, it does not provide a complete cure, and resistance eventually develops in all patients (23). Recent findings support the fact that melanoma can acquire resistance to BRAF inhibitors by multiple mechanisms. First, melanomas can rewire their signaling systems. We found that resistant melanoma cells can signal around BRAF by switching to either CRAF or ARAF to reactivate the MAPK pathway (37). Resistant melanomas can also activate tyrosine kinase receptors such as platelet derived growth factor receptor (PDGFR; ref. 38) or insulin like growth factor 1R (IGF-1R; ref. 37) to promote survival. Second, a genetic change in genes other than BRAF may also be involved in some cases (37, 38). Although Nazarian and colleagues found a NRAS mutation in a postrelapse patient sample, we noted that cells that lack PTEN are substantially less sensitive to BRAF inhibitors and that 1 postrelapse patient sample had a homozygous loss of PTEN, which was not present before treatment with PLX4032. Finally, Johannesen and colleagues identified COT as a MEK activator, driving resistance to BRAF inhibitors in cell lines and in...
patient samples (39). These findings must inform the design of future clinical trials and suggest that management of acquired resistance to BRAF inhibitors in the clinic will not be straightforward. Although identification of patient-specific resistant mechanisms may be required, a broad strategy, such as combining MEK and PI3K inhibitors (37), may prove useful. Drug combinations could also be evaluated as up-front therapy aimed at decreasing the likelihood of the emergence of resistance.

Recurrence of initially responsive melanomas may also be due to the selective outgrowth of intrinsically drug-resistant cell subpopulations (with or without secondary mutations). So far, there is no evidence that additional BRAF mutations appear as a result of BRAF inhibitor treatment (23, 37, 38). We and others have reported the existence of melanoma cell subpopulations that are phenotypically distinct from the bulk of the tumor cells, notably with respect to cycling time, growth exhaustion, and resistance to stressful environments or drug treatment. The existence of these subpopulations can be due to the activity of histone demethylases (JARID1A and JARID1B; refs. 40, 41). These findings suggest that inhibitors targeting chromatin-modifying elements or other epigenetic modifiers could be useful in combination with BRAF inhibitors to prevent resistance and eradicate all tumorigenic cell subpopulations; these studies are currently ongoing.

Although PLX4032 and other BRAF-selective inhibitors are showing efficacy for treatment of metastatic melanoma, these compounds should only be administered to patients with mutant BRAF tumors. Several recent studies have elegantly described the molecular intricacies and challenges of treating non-BRAF V600E melanomas with a BRAF inhibitor (42–46). Using different strategies and defining distinct mechanisms, together these studies show that BRAF inhibitors can induce, rather than inhibit, RAF/MEK signaling in NRAS or wild-type BRAF tumors through the formation of RAF dimers, in a RAS-dependent manner. These studies underscore the need to carefully select the right patient populations on the basis of BRAF mutational status before being included in clinical trials with BRAF-selective inhibitors.

Certainly, the issue of RAF isoform switch and complexity within the BRAF/MAPK in acquired and intrinsic resistance can be addressed by inhibiting downstream MAPK pathway effectors such as MEK and ERK. MEK inhibitors such as AZD6244, GSK1120212, and GDC-0973 (XL518) are currently under clinical investigation and could prove beneficial in a greater number of patients, or in melanomas with acquired resistance to BRAF inhibitors. However, their use in combinatorial approaches and their effects on genetically distinct patient populations still need to be carefully examined. Additionally, the availability of newer, more selective, and potent targeted agents gives us the opportunity to combine compounds to either inhibit 2 molecules within 1 pathway (e.g., BRAF and MEK) or more than 1 pathway simultaneously (e.g., MEK and PI3K). Figure 1 provides a list of compounds currently being evaluated in clinical trials, some of which are already being evaluated in combination in melanoma (http://www.clinicaltrials.gov).

Preclinical studies in models that closely mimic the human tumors, take into account both the molecular and genetic makeup of the malignant cells, and incorporate components of the microenvironment are necessary to guide the successful design of future melanoma clinical trials. Indeed, technological advances allow more drug combinations to be screened in medium- or large-scale assays in vitro, even, for example, by using 3D tumor models, in the presence or absence of stromal cells. Such screening approaches could prioritize combinations on the basis of the diverse tumors’ molecular and genetic backgrounds. Toxic effects, adverse drug interactions, and target inhibition can be more readily assessed in animal models. The emergence of more selective drugs, our better understanding of the mechanism of action of individual compounds, and the elucidation of mechanisms of drug resistance are placing us in a much better position to undertake the challenging task of rationally developing combination strategies to treat patients with advanced melanoma.

Finally, as we gain a better picture of the genetic profile of melanomas, it may be possible in the future to target other melanoma-associated oncogenes, similar to mutant BRAF, upon which the melanomas heavily rely and for which newly designed inhibitors may be effective. In the upcoming years, the Melanoma Genome Project will likely reveal a number of melanoma-relevant mutations, providing new insights into the biology and causation of this cancer and, especially, providing new therapeutic targets. We expect that this approach will lead to disease management for melanoma patients of all genetic classifications.

Conclusion

Sound scientific and clinical data based on mutant BRAF inhibitors are accumulating and indicate that targeting oncogenes in melanoma is a valid therapeutic approach. However, careful selection of patients and combination therapies are warranted, as compensatory signaling mechanisms are engaged in most melanomas sooner or later and cause resistance (acquired and intrinsic) to the compounds. Alternative treatment strategies are currently being explored and include the combination of BRAF inhibitors with other MAPK pathway inhibitors, with inhibitors targeting alternative signaling pathways, and with immunotherapies.

Disclosure of Potential Conflicts of Interest

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