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Targeted Molecular Therapy in Melanoma

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Immunotherapy and chemotherapy benefit few patients with metastatic melanoma, and even fewer experience durable survival benefit. These poor results may come from treating all melanomas as though they are biologically homogeneous. Recently, it has been shown that targeting specific activated tyrosine kinases (oncogenes) can have striking clinical benefits in patients with melanoma. In 2002, a V600E mutation of the BRAF serine/threonine kinase was described as present in more than 50% of all melanomas. The mutation appeared to confer a dependency by the melanoma cancer cell on activated signaling through mitogen-activated protein kinase pathway. The frequency and focality of this mutation (>95% of all BRAF mutations being at V600 position) suggested its importance in melanoma pathophysiology and potential as a target for therapy. The recent results of a phase 1 study with PLX4032/RG7204, a small molecule RAF inhibitor, confirm this hypothesis. Mucosal and acral-lentiginous melanomas, comprising 3% of all melanomas, frequently harbor activating mutations of c-kit and drugs targeting this mutation seem to confer similar benefits for these types of tumors. Here we provide an overview of the targeted therapy development in melanoma with emphasis on BRAF inhibition because of its prevalence and possibility of transforming the care of many melanoma patients.
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Patients with metastatic melanoma have median survivals between 6 and 10 months.[1] During the past 30 years, there has been little or no improvement in survival for these patients. Systemic therapy with immunotherapy and chemotherapy benefits only 10% to 15% of patients with metastatic disease, and even fewer experience durable benefits that impact on survival.[2,3] There is hope that by dissecting the heterogeneity of the disease, we could personalize the treatment of patients according to the specific genetic mutations (targets) present in their tumors. In other cancers, this approach has proven successful. One of the first examples was the successful development of trastuzumab for patients whose breast cancer cells had genetic amplification resulting in overexpression of the HER2/neu receptor.[4] Inhibition of abl kinase, activated in chronic myelogenous leukemia by the translocation of the B-cell receptor promoter to the coding region of abl kinase, led to the paradigm change in the therapy of this disease.[5,6] Presence of mutated CKIT gene in gastrointestinal stromal tumors leads to sensitivity to imatinib because of ability to inhibit CKIT tyrosine kinase.[7,8] More recently, mutations in the epidermal growth factor receptor (EGFR) in nonsmall cell lung cancer tumors render these tumors susceptible to EGFR tyrosine kinase small molecule inhibitors erlotinib and gefitinib.[9,10] By contrast, colorectal cancer patients with mutations in the KRAS gene are resistant to anti-EGFR monoclonal antibodies cetuximab and panitumumab and therefore should not receive these drugs.[11] Thus, if we understand better the underlying molecular changes driving individual tumors, we can conduct more efficient clinical trials in select populations and improve detection of clinical activity of new targeted agents, speeding the drug development process.

The recent advances in molecular oncology revealed the unique dependency of the cancer cells on the activated oncogene and its activated pathway (termed oncogene addiction) despite numerous redundant pathways present in normal cells. Inhibition of these activated oncogenes can mediate striking clinical benefits.[12] In 2002, investigators at the Sanger Center in the United Kingdom defined such a mutation at the V600E amino acid of the BRAF serine/threonine kinase in at least 50% of melanomas studied.[13] The mutation appeared to confer malignant properties in the cancer cell. The frequency and focality of this mutation, with 95% of all BRAF mutations at the V600E site, made it a potential target for which to develop therapeutic agents.[14,15] However, the

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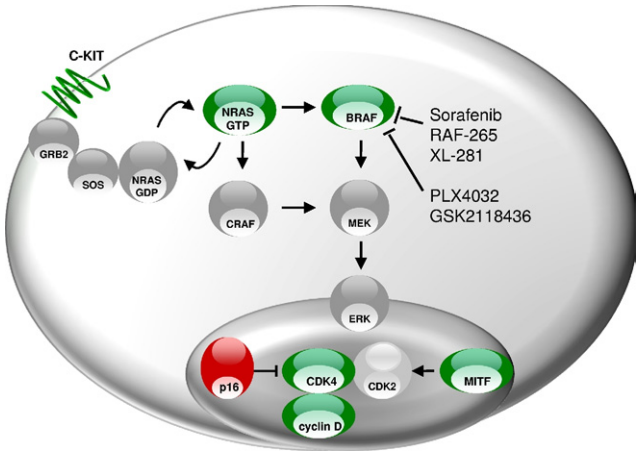

Figure 1 Genetic lesions in melanoma: targeting BRAF gain of function mutation. Multiple mutations of signaling pathways, loss of tumor suppressors, and amplification of cell cycle proteins are recurrent characteristics of melanoma cells. Targeting of V600E BRAF mutations has attracted the development of various drugs, depicted in the blue box. They have varying specificity for CRAF, BRAF, and V600BRAF.

same mutation was observed in more than 75% of benign nevi, leading to hyperplastic growth but not invasive cancer.[16,17] Therefore, there has been considerable doubt that targeting V600E BRAF mutation and its activated protein kinase gene product would lead to clinical activity in malignant melanoma, with its genetic complexity.[18-20] The last 8 years were filled with clinical trial activity that aimed to help uncover the potential for tailoring treatment to melanoma patients expressing this target.[21] The development of effective BRAF inhibitors in melanoma marks only the beginning of our attempt to target unique molecular features demonstrated by subsets of melanoma patients. The lessons learned in the process should be helpful as additional molecular targets are discovered.

Genetics of Melanoma

There are several different melanoma histologic subtypes, including superficial spreading, nodular, lentigo maligna, and desmoplastic.[22] Furthermore, melanoma can originate from the uveal tract of the eye, mucosal surfaces (oral, gastrointestinal, anorectal, and vaginal), and occasionally palms and soles (acral lentiginous), in addition to the more common cutaneous sites. Recently, a better understanding of some of the genetic heterogeneity existing among the histologic subtypes has been gained.[23-25] There are multiple activating mutations in oncogenes, including *NRAS*, *BRAF*, *C-KIT*, and *GNAQ* and *GNA11*.[23,25-27] In addition, loss of tumor suppressor genes, including *PTEN*, *P53*, and *P16* has been described, often accompanying mutated oncogenes within the same tumor (Fig. 1).[28,29] The molecular alterations appear to be linked to the histology and/or body location of the primary melanoma. BRAF mutations are found in 50% to 60% of cutaneous melanomas, most frequently in sites where sun damage is intermittent and not chronic.[23] In addition, NRAS mutations are observed in 15% to 30% of cutaneous melanomas and are mutually exclusive of BRAF mutations.[26,28] This confers constitutive activation of the MAP kinase pathway in approximately 75% of all cutaneous melanoma cases. The CDKN2A gene products, p16 and p14 cell cycle regulators, are frequently lost or nonfunctional, especially in tumors arising from chronically sun damaged skin.[23,30] CKIT mutations have been most frequent (15%-20%) in acral and mucosal melanomas.[24,25] Mutations in GNAQ and GNA11 are mutually exclusive and found in more than 80% of uveal melanomas.[27] These mutations are potential targets for therapy through blockade of the mutated proteins or other signaling molecules downstream in the same signaling pathways.

B-RAF Inhibitors: Targeted Therapy in Malignant Melanoma

The advances in molecular genetics of melanoma led to the identification of BRAF as a potential target for melanoma therapy. BRAF is part of the RAF family of kinases, including A-RAF, B-RAF, and C-RAF signaling molecules in the MAP kinase pathway. These molecules transmit extracellular signals from the cell membrane to the nucleus via a cascade of phosphorylation events (RAS/RAF/MEK/ERK) and ultimately lead to growth signals, resulting in tissue invasion, metastasis, apoptosis evasion, cell growth and survival, differentiation and senescence, vascular development, and angiogenesis.[29,30] The general importance of this pathway for cancer development is suggested by the finding of BRAF mutations in 6% to 8% of all human cancers.[31] The most common mutation in melanoma is in the BRAF kinase domain, exon 15. A single-base missense mutation (T → A) at codon 1799 leads to substitution of glutamic acid for valine at position 600 of BRAF protein (V600E). This "gain-of-function" BRAF mutation accounts for more than 90% of the BRAF mutations in melanoma.[32] This acquired, somatic, noninherited activating mutation leads to kinase activity which is many times higher than occurs in normal cells leading to downstream extracellular signal-regulated kinase (ERK) signaling and driving cell transformation, allowing the cells to proliferate in a growth factor–independent fashion.[13,14]

BRAF Inhibition in the Clinic

Although BRAF is an attractive target, initial agents thought to be targeting this mutated protein lacked clinical efficacy. This may have been the result of the agent's poor activity against the V600E mutated BRAF, or the limitations in the effective drug concentrations that could be achieved due to the toxic effects of inhibiting other signaling molecules beyond RAF. Fortunately, the specificity and efficacy of the more recent BRAF inhibitors have improved over the past few years (Fig. 1). Advancing beyond the efficacy observed with these agents will require a better understanding of the targeted pathways and mechanisms of resistance. Carefully designed strategies to optimize combinations and sequences of these agents, potentially including chemotherapy and immu-

notherapy, may ultimately lead to the cure of this particular subtype of melanoma.[32]

Sorafenib as the First RAF Inhibitor in Clinic: an Example of the Pitfalls in Oncogene-Targeted Therapy

The first BRAF inhibitor to be investigated extensively in clinical trials of melanoma was sorafenib (BAY 43-9006 or Nexavar; Bayer Pharmaceuticals, Montvale, NJ). The agent was developed primarily with the intent to block CRAF, which was known to be downstream from activated, oncogenic RAS proteins. There was minimal, if any, clinical activity with single-agent sorafenib in advanced melanoma, with few patients experiencing even disease stabilization.[33] Melanomas were not prospectively tested for V600E BRAF mutations based on the assumption that RAS or BRAF mutations could activate the pathway and thus possibly confer sensitivity. Retrospective analysis has not uncovered any detectable difference in outcome among patients with BRAF, NRAS or neither mutation. Phase 2 clinical trials of sorafenib in combination with chemotherapy (carboplatin plus paclitaxel, or dacarbazine) looked promising, with increased response rates and longer than previously seen progression-free survivals.[34] However, there was no difference in outcomes between wild-type or mutated BRAF subgroups. Phase 3 trials in both front-line and second-line therapy in combination with carboplatin plus paclitaxel failed to demonstrate improvement in survival.[35,36]

This ended the development of sorafenib in melanoma, whereas the drug was approved for renal cell cancer because of inhibitory activity for VEGFR2, PDGFRα, and PDGFRβ.[37] Sorafenib does not inhibit the mutated BRAF protein with high specificity; therefore, it did not provide a true test of the therapeutic value of mutated BRAF inhibition. However, because of CRAF inhibitory activity, sorafenib may still prove to be useful, alone or in combination, when resistance develops to V600E BRAF inhibitors.[38] The identification of a new class of BRAF inhibitors that contain pyridoimidazolone as the ATP hinge-binding domain and a rigid imidazolone group represented a basis for development of more selective inhibitors.[39] Building upon this novel scaffold, drug candidates with inhibitory potency against mutated BRAF in isolated kinase assays and in cellular assays were developed.

Novel BRAF Inhibitors in Development

RAF-265 is a novel, orally active agent that potently inhibits all RAF isoforms (A-RAF, B-RAF, C-RAF), including BRAF V600E. It also inhibits VEGFR-2, c-KIT, and PDGFRβ.[40] It targets BRAF V600E and vascular endothelial growth factor receptor-2 most potently in cell-based assays. RAF-265, previously known as CHIR-265 (Novartis Pharmaceuticals, Basel, Switzerland), shows biochemical IC_{50} values of <100 nM against BRAF-mutant melanoma cell lines (eg, 50-fold to 100-fold more potent than sorafenib in the same genetic context). RAF-265 has been shown to induce tumor regression and has antiangiogenic effects in animal models. It is currently in phase 1 clinical trials to determine the maximum tolerated dose (MTD), dose-limiting toxicities, the safety profile when given to patients with locally advanced or malignant melanoma, and ability to inhibit BRAF signaling at tolerable doses.

XL281 is a novel small molecule designed to selectively inhibit RAF kinases that displays high oral bioavailability in multiple preclinical species and strongly inhibits RAS/RAF/MEK/ERK signaling in human xenograft tumor models.[41] This action translates into substantial inhibition of tumor growth in preclinical models of human tumors that overexpress receptor tyrosine kinases or harbor activating mutations in RAS or RAF. XL281 exhibited growth-inhibitory antitumor activity in human xenograft tumor models driven by activating mutations in BRAF or KRAS. In a phase 1 trial, 29 patients, including 7 with colorectal cancer, 5 with papillary thyroid cancer, and 4 with melanoma, were enrolled in 7 cohorts, with XL281 administered orally at doses ranging from 10 to 225 mg daily.[41] XL281 was generally well tolerated, with dose limiting toxicity observed at 225 mg and MTD of 150 mg daily. One subject with ocular melanoma achieved a partial response, and 12 patients with stable disease ($\geq$3 months) have been observed. Five patients, 3 with papillary thyroid cancer and 2 with a confirmed BRAF V600E mutation, had stable disease (68, 64+, 53+, 26, and 20 weeks, respectively). In pharmacodynamic analyses, substantial modulation of the BRAF signaling pathway was observed in tumor tissue, skin, and hair, as indicated by decreases in the phosphorylation of MEK and ERK following treatment with XL281. A reduction in proliferation and an increase in apoptosis were observed in tumor tissue following treatment with XL281. Further evaluation of XL281 is ongoing at MTD expansion cohorts for colorectal cancer, papillary thyroid cancer, melanoma, and nonsmall cell lung cancer.

GSK2118436, highly related to SB-590885 (GlaxoSmithKline Pharmaceuticals, Philadelphia, PA) is a novel molecule that selectively inhibits RAF kinases with more potency toward BRAF than CRAF.[42] The structural basis of BRAF inhibition by SB-590885 is due to stabilization of the active conformation of BRAF by SB-590885, in contrast to stabilization of the inactive conformation of BRAF by sorafenib. Therefore, it may have the potential to overcome resistance to inhibitors that bind to the inactive conformation of BRAF, as has been shown for other oncogene-dependent malignancies. SB-590885 inhibits BRAF kinase enzymatic activity 100-fold more potently than sorafenib, BAY 43-9006.[42] SB-590885 showed preferential inhibition of biochemical signaling, proliferation, survival, and transformation in human tumor cell lines expressing oncogenic BRAF. This clinical candidate, GSK2118436, is completing phase 1 studies with plans for Phase 2/3 trials both as a single agent and in combination with MEK inhibitor (glycogen synthase kinase 1120212).[43,44]

Proof of Principle for BRAF Inhibition: PLX4032/RG7204

Tsai and colleagues[39] developed a selective inhibitor of active V600E mutated BRAF using a structure-guided discovery approach. PLX4720, a 7-azaindole derivative, defines a class of kinase inhibitors with marked selectivity in both biochemical and cellular assays. Potent cytotoxic effects are limited to cells with this specific mutation leading to inhibition of ERK phosphorylation. Melanoma animal models have shown that PLX4720 induces cell cycle arrest and apoptosis in the BRAF V600E mutated xenografts without significant toxicity.[39] Therapeutic testing in human trials was conducted with PLX4032, a drug similar in structure to PLX4720. PLX4032 is a novel oral small molecule with selective inhibition of the oncogenic V600E mutant BRAF kinase among 70 kinases screened with preclinical in vivo and in vitro activity (submitted for publication).

Data presented at the 2009 American Society of Clinical Oncology meeting showed unprecedented antitumor activity in V600E BRAF mutant tumors in phase 1 dose-escalation study.[45] Fifty-five patients were enrolled, including those with metastatic melanoma (n = 49), thyroid (n = 3), rectal (n = 1), or ovarian carcinoma (n =1). Dose-limiting toxicities included fatigue, myalgias, and rash in 3 of 5 patients at 1120 mg twice daily. Squamous cell carcinoma of the skin, keratoacanthoma type, emerged in 23% of patients within the first several months of therapy. Doses at or greater than 240 mg orally twice daily with an optimized formulation were able to deliver blood levels in or well above the minimum effective concentration in preclinical models. Results in the V600E BRAF melanoma patients (n = 16) at doses >240 mg orally twice daily showed 10 partial responses and one complete response, with 9 being confirmed with regression of liver, lung, and bone metastasis. Positron emission tomography results from baseline and at day 15 showed remarkable reduction in glucose uptake in most patients. Some patients had symptomatic improvement within days of starting treatment. The MTD was selected at 960 mg twice daily. Median progression-free survival in this cohort was approximately 8.5 months.[45]

An extension cohort of 32 melanoma patients treated at the MTD of 960 mg twice daily was then enrolled.[46,47] The median age was 52 years, 74% having M1c disease, 45% Eastern Cooperative Oncology Group (ECOG) performance status 0%, 55% ECOG performance status 1%, and 39% given 3 or more previous therapies for metastatic disease. Drug-related toxicities were seen in more than 10% of the extension cohort and included rash (68%), arthralgias (48%), photosensitivity (42%), fatigue (32%), cutaneous squamous cell carcinoma (23%), pruritus (23%), palmar-plantar dysesthesia (23%), nausea (19%), alopecia (16%), and hyperbilirubinemia (13%). To date, this cohort of V600E BRAF-mutated melanoma patients treated with PLX4032 at MTD of 960 mg twice daily showed an overall Response Evaluation Criteria in Solid Tumors objective response in 26/32 patients (81%), with 2 complete responses compared with a historical experience of 10%-15% objective response rate with conventional drugs. Toxicity was manageable at 960 mg twice daily. The median progression-free survival has been 8.0 months, with median overall survival not yet reached.

A phase 2 trial of PLX4032 in 120 previously treated V600E BRAF-mutated patients has finished accrual in the United States and Australia. The objective response rate was selected as the primary end point. A phase 3 randomized trial of PLX4032 or dacarbazine chemotherapy as first-line therapy in 500 patients with V600E-mutated melanoma has opened worldwide in January 2010 with overall survival as primary endpoint.

The development of cutaneous squamous cell carcinomas, keratoacanthoma type, occurred in up to 30% of patients treated at the phase 2 dose. They were mostly easily treated with local therapy, have not shown any metastatic potential and, in a few instances, regressed upon dose interruption. Interestingly, both sorafenib and XL281 have induced similar skin lesions, though at a lower rate. Recent published data demonstrate that selective BRAF inhibitors can activate the MAP kinase pathway in vitro in cells that lack a BRAF mutation.[47-50] This finding may pertain to some of the toxicities observed with PLX4032, including the emergence of these cutaneous squamous cell carcinomas. Ongoing studies should bring insights to the molecular pathways active in development of these skin lesions and potentially help in development of the next generation of BRAF inhibitors.

Conclusions

Recent data with PLX4032 provide the first evidence that therapy targeting tumors containing activating BRAF-V600E mutations can induce significant tumor regression in patients with melanoma. This represents the first example of successful targeting of an intracellular signaling molecule that harbors an activating mutation. The ongoing phase III trial should provide an answer whether this approach leads to improved overall survival in comparison with standard chemotherapy.

However, despite impressive clinical successes with the selective kinase-targeted therapies, most treatment-responsive patients ultimately relapse because of acquired resistance.[51,52] Our greatest challenge lies in the elucidation of mechanisms by which resistance develops which may lead to a rational basis for combination therapy or second-generation drugs to combat this resistance. Lastly, investigations of the BRAF inhibitors in the adjuvant setting may provide the greatest impact on the outcome of melanoma patients whose tumors harbor BRAF mutations if eradication of microscopic residual disease following surgery can be achieved (Fig. 1).

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