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RAF KINASE INHIBITORS FOR THE TREATMENT OF MELANOMA

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SUMMARY

Malignant melanoma is one of the most lethal solid tumors unless detected early. Malignant melanoma accounts for about 75% of skin cancer-related deaths. Around 68,700 cases of new melanoma were diagnosed in 2009 in the U.S. According to a World Health Organization report, about 48,000 melanoma-related deaths occur worldwide per year. In its early stages, melanoma can be treated surgically, leading to 5-year survival rates exceeding 90%. However, when it becomes metastatic, it is uniformly fatal, with 5-year survival rates of < 2% and a median survival of 6-10 months. Recent advances in the understanding of the molecular heterogeneity offer the promise of a better future for drug development in melanoma. The MAP kinase pathway is known to be associated with several human cancers, including malignant melanoma. The MAP kinase pathway is activated through mutations in BRAF, and BRAF V600E is the most common among these mutations. Targeted therapy involving B-raf inhibition was thought to yield promising results. This review provides information on various B-raf inhibitors and the clinical impact these drugs may have in patients with melanoma.

INTRODUCTION

Malignant melanoma is one of the most lethal solid tumors unless detected early. Malignant melanoma accounts for about 75% of skin cancer-related deaths. Around 68,700 cases of new melanoma were diagnosed in 2009 in the U.S. (1). According to a World Health Organization report, about 48,000 melanoma-related deaths occur worldwide per year. In its early stages, melanoma can be treated surgically, leading to 5-year survival rates exceeding 90%. However, when it becomes metastatic, it is uniformly fatal, with 5-year survival rates of < 2% and a median survival of 6-10 months. Despite unrelenting efforts at developing new drugs, over the past 30 years there has been little or no improvement in the overall survival for patients with metastatic disease. Recent advances in the understanding of the molecular heterogeneity of the disease offer the promise of a better future for drug development in melanoma. Elucidation of the molecular mechanisms of carcinogenesis in melanoma has expanded the horizon of opportunity to alter the natural history of the disease. Multiple signal transduction pathways appear to be aberrant and drugs that target them are in development. In this review, we will discuss the current status of drug development, with a focus on Raf kinase inhibitors.

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THE TARGET: MAP KINASE PATHWAY

Extensive research performed in the last few years has helped us better understand the signal transduction pathways involved in human cancers. The MAP kinase pathway has long been associated with human cancers (2). This pathway regulates cell growth, survival, differentiation, metastasis, senescence and apoptosis. The Raf kinase family includes A-Raf, B-raf and c-RAF, which are intermediate molecules in the Ras/Raf/MEK/ERK serine/threonine-protein kinase pathways. These molecules sequentially relay proliferative signals generated at cell surface receptors and through cytoplasmic signaling into the nucleus via a cascade of phosphorylation events.

The MAP kinase pathway is found to be activated through *BRAF* mutations in 6-8% of all human cancers. Mutations in *BRAF* are found in about 50-70% of cutaneous melanomas (3), and *BRAF* V600E is the most prevalent. This is a single-base missense mutation with substitution of glutamic acid for valine at position 600 of the V600E (2, 3). Acquired somatic V600E mutated *BRAF* leads to kinase activity about 10-fold higher than in normal cells. This increased activity subsequently causes unchecked stimulation of MAP kinase/ERK signaling driving cell transformation, activation of antiapoptotic and angiogenic pathways, allowing the cells to proliferate in a growth factor-independent fashion, and ultimately, enhanced growth and progression of melanoma. V600E mutated *BRAF* shows an increased sensitivity to MEK inhibition and results in more potent tumor cell growth arrest than cell lines not expressing

the V600E mutation. Regardless of *BRAF* V600E mutational status, virtually all melanomas are associated with activity in the MAP kinase pathway.

SORAFENIB

Target specificity

Sorafenib (Bayer/Onyx) is FDA-approved for the treatment of advanced primary renal cell carcinoma and advanced primary hepatocellular carcinoma. It is an oral multikinase inhibitor that is not highly specific for the MAP kinase pathway; it inhibits tumor growth by acting on tumor cells and cells of the tumor vasculature. Sorafenib also potently inhibits vascular endothelial growth factor receptor VEGFR-1, -2 and -3, and platelet-derived growth factor receptor (PDGFR) (4, 5). Sorafenib's multiple targets, including wild-type *BRAF*, oncogenic *BRAF* V600E and proangiogenic receptor tyrosine kinases, enable its action on tumor cells and tumor vasculature to induce apoptosis, inhibit proliferation, as well as angiogenesis, in preclinical models. This provides sorafenib with the potential for activity against a wide variety of tumor types and may also provide a means to overcome multidrug resistance. In fact, it has been proposed that sorafenib may sensitize tumor cells to chemotherapy through downregulation of antiapoptotic molecules. It may also enhance the delivery of cytotoxic agents by affecting the vasculature, thereby increasing the effectiveness of concomitantly administered chemotherapy.

Clinical development

Sorafenib was tested in several phase I-III clinical trials. The agent can be administered conveniently by the oral route. Single-agent sorafenib 400 mg b.i.d. was found to be generally well tolerated. The adverse effects were mostly mild to moderate in severity, had a predictable course and were readily manageable. The most frequently observed drug-related adverse events were dermatologic (hand–foot skin reaction, rash/desquamation), diarrhea and fatigue (6, 7). Severe biochemical abnormalities, myelosuppression, hematologic, cardiovascular, hepatic and renal toxicities were seldom reported (8). Treatment-emergent hypertension was observed in 5-17% (5% grade 3 or 4) of patients receiving sorafenib 400 mg b.i.d. and was readily manageable with antihypertensive agents.

A phase II trial of sorafenib in metastatic melanoma aimed to determine if treatment with sorafenib altered tumor proliferation and induced antitumor responses in wild-type or *BRAF* mutated melanoma. Sorafenib 400 mg p.o. b.i.d. was given on days 1-28 every 4 weeks. Repeat biopsies were obtained on day 28 for KI-67, cyclin D1 and ERK, serum collagen cryptic epitopes, and reimaging was done every two cycles. Patients were treated until progression. Follow-up analysis was done on completion of the trial and 37 patients were enrolled. Routine *BRAF* sequencing yielded 32 wild-type and 5 mutant samples. MS-PCR yielded 22 wild-type and 15 mutant samples. Toxicity was identical in both groups. In patients with mutant *BRAF*, one partial response and four cases of progressive disease were noted. In patients with wild-type *BRAF*, 2 partial responses, 6 stable diseases and 12 cases of progressive disease were noted after 2 cycles. Assessment was incomplete in 12 cases. Epitopes decreased with responses lasting 16-32 weeks; biopsies demonstrated downregulation of tumor KI-67, ERK and cyclin D1 in cases with partial response and stable disease. This study showed that sorafenib monotherapy is minimally active, with few short-term responses in both mutant and wild-type *BRAF* malignant melanoma. Downregulation of proliferation markers was demonstrated and MS-PCR improved the detection of mutant *BRAF* (9).

A randomized phase II discontinuation trial analysis was done to evaluate the effects of sorafenib in patients with advanced melanoma. Enrolled patients received a 12-week run-in of sorafenib 400 mg b.i.d. Patients with changes in bidimensional tumor measurements of < 25% from baseline were then randomized to sorafenib or placebo for a further 12 weeks. Patients with ≥ 25% tumor shrinkage after the run-in continued on open-label sorafenib, whereas those with ≥ 25% tumor growth discontinued treatment. Of 37 melanoma patients treated during the run-in phase, 34 were evaluable for response: 1 had ≥ 25% tumor shrinkage and remained on open-label sorafenib, 6 (16%) had < 25% tumor growth and were randomized (placebo, n = 3; sorafenib, n = 3), and 27 had ≥ 25% tumor growth and discontinued therapy. All three randomized sorafenib patients progressed by week 24; one remained on sorafenib for symptomatic relief. All three placebo patients progressed by week 24 and were restarted on sorafenib; one experienced disease restabilization. Overall, the confirmed best responses for each of the 37 melanoma patients who received sorafenib were: 19% stable disease, 62% (n = 23) progressive disease and 19% (n = 7) unevaluable. The overall median progression-free survival (PFS) was 11 weeks. The 6 randomized patients with stable disease had overall PFS values ranging from 16 to 34 weeks. In conclusion, sorafenib is well tolerated but has little or no antitumor activity in advanced melanoma patients as a single agent at the dose evaluated (400 mg b.i.d.) (10).

The effects of the combination of paclitaxel, carboplatin and sorafenib were investigated in a phase I/II trial in 35 patients with progressive stage IV melanoma pretreated with no more than 3 previous chemotherapy regimens. The preliminary results showed a high rate of partial responses (40%) and stable disease (43%), but antitumor activity was independent of *BRAF* mutational status. Responses were observed mainly in patients with skin, subcutaneous and lymph node metastases (stage M1a) on a limited number of previous therapies. This led to two large multicenter, randomized, placebo-controlled phase III trials investigating the addition of sorafenib to a carboplatin and paclitaxel backbone in both first- and second-line therapy of metastatic melanoma. Both trials have been concluded.

The PRISM trial was a randomized, double-blind, placebo-controlled phase III study conducted to evaluate the efficacy and safety of sorafenib with carboplatin and paclitaxel in patients with advanced melanoma who had progressed on a dacarbazine- or temozolomide-containing regimen. About 270 patients were randomly assigned to receive paclitaxel 225 mg/m^2 i.v. plus i.v. carboplatin at area under the curve 6 (AUC 6) on day 1 of a 21-day cycle, followed by either placebo (n = 135) or oral sorafenib 400 mg (n = 135) twice daily on days 2-19. The primary efficacy endpoint was PFS; secondary and tertiary endpoints included overall survival and incidence of best response, respectively. The median PFS was 17.9 weeks for the placebo plus carboplatin and paclitaxel arm and 17.4 weeks for the sorafenib plus carboplatin and paclitaxel arm (hazard ratio

[HR]: 0.91; *P* = 0.49). The response rate was 11% with placebo versus 12% with sorafenib. In this study, the addition of sorafenib to carboplatin and paclitaxel did not improve any of the endpoints over placebo plus carboplatin and paclitaxel in the second-line setting for patients with advanced melanoma (11).

A double-blind, randomized phase III trial compared carboplatin/paclitaxel with or without sorafenib in the first-line therapy of metastatic melanoma. The primary objective was to determine whether addition of sorafenib improved overall survival compared to carboplatin and paclitaxel in chemotherapy-naive metastatic melanoma patients. Patients were not prescreened for *BRAF* mutations. The same dose and schedule used for PRISM were used in this trial. A total of 823 patients (411 on carboplatin/paclitaxel, 409 on sorafenib/carboplatin/paclitaxel; 3% ineligible) were accrued over 34 months. After the third interim analysis, 75% of the events required for final analysis had transpired. The study had crossed the futility boundary and was unblinded. The log-rank test HR, stratified by American Joint Committee on Cancer (AJCC) stage, ECOG Performance Scale and prior therapy, was 1.0 (*P* = 0.878). The median overall survival for the sorafenib plus carboplatin and paclitaxel group was 11.1 months and for the carboplatin and paclitaxel group 11.3 months. Median PFS was 4.9 for sorafenib plus carboplatin and paclitaxel and 4.1 for carboplatin and paclitaxel. Toxicities were comparable in both groups. Sorafenib did not improve overall survival in combination with carboplatin and paclitaxel in chemotherapy-naive patients with metastatic melanoma (12).

In a phase I/II clinical trial of sorafenib in combination with temozolomide in patients with metastatic melanoma, the maximum tolerated dose (MTD) was found to be 400 mg sorafenib p.o. b.i.d. and 150 mg/m^2 temozolomide given p.o. for 7 days every other week. Sixty-three patients with metastatic melanoma were included in the dose-escalating phase I (n = 15) and the phase II (n = 48) parts. Half of them had received ≥ 2 lines of prior chemotherapies and 37% had high lactate dehydrogenase. The median number of cycles was 4 (1-22). Toxicity was manageable and required treatment interruption for only three patients. Of 49 evaluable patients, 4 had a partial response (8%), 19 had stable disease (39%) and 26 had progressive disease (53%), for a treatment benefit in 47%. Six-month PFS and overall survival rates were 15.2% and 60.6%, respectively. Of 37 tumors tested, mutations were found in *BRAF* (62%), *STK11* (25%), *NRAS* (8%), *TP53* (6%) *CDKN2A* (3%) and *CTNNB1* (3%), without a correlation to clinical response (13).

Sorafenib has been evaluated in combination with temozolomide, an oral alkylating agent widely used for the treatment of patients with metastatic melanoma with or without brain metastases. Results from a four-arm phase II trial demonstrated encouraging antitumor activity and tolerability for this combination in patients with metastatic melanoma. About 167 patients were enrolled. The results show that temozolomide plus sorafenib was well tolerated and displayed activity in melanoma patients without a prior history of temozolomide treatment. The activity of this combination regimen warrants further investigation (14).

Sorafenib was also evaluated in combination with dacarbazine (DTIC) in a single-center, open-label, dose-escalation phase I trial in patients with metastatic melanoma. Among 18 evaluable patients, 3 (17%) had a partial response and 11 (61%) had stable disease. This combination was further evaluated in clinical trials, including an open-label, first-line, uncontrolled phase II study, as well as a randomized, placebo-controlled phase II study in patients with unresectable stage III or IV melanoma. In the uncontrolled phase II study, sorafenib and DTIC were well tolerated and yielded promising efficacy results in these patients with a poor prognosis. Eight patients (10%) achieved a partial response and 34 (41%) had stable disease; the median PFS was 14 weeks and the median overall survival time was 41 weeks. These data were encouraging compared with DTIC alone, which achieved a response rate of 7.5% and a PFS of 6 weeks. Results from a placebo-controlled study support a better efficacy trend in terms of objective responses and PFS compared with DTIC alone in advanced melanoma. The median PFS times were 21.1 weeks versus 11.7 weeks , respectively, for sorafenib in combination with DTIC compared with DTIC plus placebo (10).

Melanoma is driven by multiple genetic lesions that disrupt signaling through several pathways; these pathways are embedded within complex networks that influence each other's activity, and it is likely that effective treatments will require simultaneous targeting of several pathways. It may even be necessary to target a single pathway at multiple points to achieve effective treatment. The redundancy within the multiple signaling pathways activated in melanoma, along with the likelihood of drug resistance, suggests that combination therapy strategies will be required for effective disease management.

A prime example of a targeted therapy combinatorial approach was the Southwest Oncology Group Trial S0438, which was a randomized phase II trial of sorafenib with temsirolimus or tipifarnib as first-line therapy for advanced metastatic melanoma. Temsirolimus is an inhibitor of the mTOR pathway that could potentially be upregulated in response to MAP kinase pathway upstream inhibition, while tipifarnib is a farnesyltransferase inhibitor that prevents the activation of Ras oncogenes, inhibits cell growth, induces apoptosis and inhibits angiogenesis. The study was performed to assess the objective response rate and PFS in patients with previously untreated non-ocular metastatic melanoma. The patients were randomized to sorafenib 200 mg p.o. b.i.d. plus temsirolimus 25 mg i.v. weekly (arm A), or sorafenib 400 mg p.o. every morning and 200 mg p.o. every evening continuously plus tipifarnib 100 mg p.o. on days 1-21 every 28 days in 2-stage accrual (arm B). After the first 30 patients in each arm, ≥ 3 responses or ≥ 9 patients progression-free at > 4 months were required to proceed to stage 2, adding 25 patients. Dose adjustments (one or both drugs) were based on reported agent-specific toxicities. In stage 1, 48 patients enrolled in arm A, 45 evaluable for PFS and 44 for response. In arm A, a stage I partial response was seen in 2 patients and 4-month PFS in 11 patients. Arm A then accrued to stage 2 a total of 67 patients; the final results have not been reported. In stage I, 42 patients were enrolled in arm B; 41 were evaluable for PFS and response, with 1 partial response and 4-month PFS in 8 patients. Arm B was then closed. Serious toxicities occurred in one patient in each arm. Combinations appear to show insufficient activity, possibly due to poor pathway inhibition at tolerated doses (15).

While the development of sorafenib has been fraught with difficulties, in some respects related to selectivity, novel B-raf kinase inhibitors that are selective for the oncogenic V600E mutant are showing exciting results.

VEMURAFENIB

Target specificity

Vemurafenib (PLX-4032, RG-7204; Plexxikon/Roche) is a novel oral small molecule that selectively inhibits the oncogenic V600E mutant B-raf kinase among 70 kinases screened, with preclinical in vivo and in vitro activity. Potent cytotoxic effects are limited to cells with this specific mutation, leading to inhibition of ERK phosphorylation.

Clinical development

A dose-escalation phase I study of vemurafenib was designed to determine the MTD, safety, pharmacokinetics/pharmacodynamics and efficacy (RECIST evaluation every 8 weeks) in sequential cohorts of three to six patients. Fifty-four patients were enrolled with metastatic melanoma (n = 49), thyroid (n = 3), rectal (n = 1) or ovarian carcinoma (n = 1). Twenty-six patients received a crystalline formulation continuously at doses of 100-1600 mg b.i.d., with associated exposures below target plasma levels. Twenty-eight patients received an optimized formulation with increased bioavailability, predicted to have 10-fold greater bioavailability, at doses of 160-1120 mg b.i.d. Thirteen melanoma patients (77% M1C) treated at doses of 240 mg b.i.d. or higher of the increased bioavailability formulation had a minimum follow-up of 8 weeks. Five of the seven *BRAF* V600E$^+$ patients treated at > 240 mg b.i.d. had tumor regression, or up to 83%, with one confirmed and one unconfirmed partial response (too early); two of four patients with unknown V600E status had tumor regression, or up to 50%, with one confirmed partial response; two *BRAF* wild-type patients had progressive disease. All 7 patients with tumor regression remained progression-free for 4-14 months. Three thyroid cancer patients with V600E mutations had tumor regression (range: 9-16%) and were progression-free (4-7 months). Vemurafenib exhibits antitumor activity in V600E *BRAF* mutant tumors. These observations confirm that V600E *BRAF* is a valid therapeutic target in human cancer. The MTD was 960 mg b.i.d. Dose-limiting toxicities were observed at 1120 mg b.i.d. Drug-related toxicities were seen in > 10% of the extension cohort and included rash (68%), arthralgias (48%), photosensitivity (42%), fatigue (32%), cutaneous squamous cell carcinoma (keratoacanthoma type; 23%), pruritus (23%), palmar–plantar dysesthesia (23%), nausea (19%), alopecia (16%) and hyperbilirubinemia (13%) (16).

An international, multicenter phase I study was performed to determine the early FDG-PET responses to vemurafenib in *BRAF* mutant advanced melanoma. FDG-PET is an effective imaging modality for assessing early response to a variety of agents that target key driver mutations in human cancer. Reduction in FDG uptake reflects inhibition of glycolytic metabolism, and presumably rapid cell death, and thus may provide early information on patient benefit. Baseline and day 15 FDG-PET was available in 22 patients with advanced metastatic melanoma treated at 320 mg b.i.d. The cohort included 19 patients treated with at 960 mg b.i.d. vemurafenib. Four patients with advanced melanoma treated in the same study but at subtherapeutic doses were analyzed. Strikingly, all 22 patients treated at 320 mg b.i.d. had at least a partial metabolic response, with 3 achieving a complete metabolic response. In the 19 patients with partial metabolic response there was an 84 ± 3% reduction in target lesion standard uptake value (SUV_{max}), a 90 ± 4% decrease in the % of injected dose (%ID) in all identified disease sites, and a decrease in metabolic volume of 79 ± 5%. In contrast, no metabolic responses were seen in the four control subjects. The overall RECIST response rate in the cohort of 22 patients was 86%, with 17 partial responses and 2 complete responses. There appeared to be no relationship between either SUV_{max} or metabolic tumor burden at baseline and extent of PET response. There was a positive correlation between %ID in all identified diseases and %ID in target lesions (r^2 = 0.52; P = 0.002), indicating significant homogeneity of response between lesions in individual patients. No relationship was found between reduction in target lesion SUV_{max} and response by RECIST, PFS or time to achieve RECIST partial response. FDG-PET is a useful marker of early biologic response in *BRAF* mutant melanoma treated with vemurafenib. Little heterogeneity in PET response was found between lesions in individual patients, suggesting minimal intrapatient molecular heterogeneity with respect to effects of B-raf inhibition in patients with metastatic melanoma (17).

Clinical and histologic characteristics of vemurafenib therapy-associated cutaneous neoplasms were studied in a phase I trial. Vemurafenib is associated with the development of skin lesions, including keratoacanthoma and rarely squamous cell cancer, as confirmed by central review. Overall, these are well-differentiated neoplasms with likely a low probability of invasive and metastatic potential. Frequent dermatological and head, neck and chest evaluations are ongoing for all patients in vemurafenib clinical trials (18).

Recently, a phase II trial of vemurafenib in V600E *BRAF* mutated patients completed accrual in the U.S. and Australia. The open-label, multicenter study enrolled 132 patients. As of September 27, 2010, data showed a confirmed response rate of 52%, including 3 confirmed complete responses, 66 confirmed partial responses and 39 patients with stable disease. The median PFS was 6.2 months compared to a historical PFS of < 2 months. The median duration of response was 6.8 months. Median overall survival has not yet been reached (19).

A randomized phase III trial of vemurafenib compared with dacarbazine chemotherapy in V600E *BRAF* mutated melanoma opened worldwide beginning in December 2009. Numerous challenges still need to be addressed.

Recent reports in the literature indicate that the incidence of *BRAF* V600K mutations in melanoma patients is higher than is commonly assumed. Despite the fact that it can be present in up to 10% of all melanoma cases, patients with *BRAF* V600K mutations are currently excluded from clinical trials with vemurafenib. Preclinical data have demonstrated similar kinase activity for the V600K and V600E mutations, together with clear evidence of clinical activity for vemurafenib in a patient with a documented V600K mutation, suggesting that melanoma patients with V600K mutations may need to be included in current and future trials of B-raf inhibitors (20).

XL-281

Target specificity

XL-281 (Exelixis/Bristol-Myers Squibb) 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 tumor xenografts. This

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*. XL-281 exhibited antitumor activity in human tumor xenograft models driven by activating *BRAF* or *KRAS* mutations.

Clinical development

Patients with advanced solid tumors were enrolled in a phase I study in successive cohorts treated with XL-281 p.o. once daily on a 28-day cycle. Tumor response was assessed using RECIST every 8 weeks. Plasma pharmacokinetic and pharmacodynamic samples were collected. The MTD was expanded to 10 patients each with melanoma, colorectal, papillary thyroid and non-small cell lung cancer (NSCLC). Pre- and post-dose tumor and surrogate tissues were obtained. Biomarker and genotype analyses of pathway genes were performed. The dose-escalation phase is complete; 30 patients were treated with XL-281. One patient with an ocular melanoma demonstrated a partial response of 4 months' duration. Twelve patients had stable disease (3-17+ months), including 2 with I^{131}-refractory papillary thyroid cancer harboring *BRAF* V600E mutations (15+ and 17+ months). Nine of these patients had decreases in target lesions (5-29%), including a patient with *KRAS* mutant colorectal carcinoma on study for 20 weeks with marked symptomatic improvement. The MTD of XL-281 was 150 mg and it was generally well tolerated at this dose. Dose-limiting toxicities of fatigue, nausea, vomiting and diarrhea were observed at a dose of 225 mg. The most common adverse events included grade 1/2 fatigue (48%), diarrhea (35%), nausea (35%), vomiting (35%) and anorexia (30%). Three patients had related adverse events ≥ grade 3: hypokalemia, nausea and vomiting. At the MTD, paired biopsies from four patients (three melanoma, one NSCLC) showed an average 72% decrease in pMEK, 68% decrease in pERK, 24% decrease in KI-67 (proliferation) and 64% increase in TUNEL (apoptosis). Three of six evaluable patients in the MTD cohort showed stable disease at first assessment, including one melanoma patient with an *NRAS* Q61R mutation who showed a 20% decrease in target lesions. One partial response occurred in a subject with ocular melanoma, and clinical benefit (partial response or stable disease) occurred in 43% (13/30) of patients in the dose-escalation phase. A reduction in proliferation and an increase in apoptosis were observed in tumor tissue following treatment with XL-281. Further evaluation of XL-281 is ongoing in an MTD expansion including colorectal cancer, papillary thyroid cancer, melanoma and NSCLC (21).

GSK-2118436

Target specificity

GSK-2118436 (GlaxoSmithKline) is a highly potent and selective ATP-competitive B-raf inhibitor with > 100-fold selectivity for mutant B-raf over wild-type B-raf in cell lines. It displays concentration-dependent inhibition of MEK and ERK phosphorylation in mutant B-raf cell lines and tumor regression in xenograft models. The structural basis for B-raf inhibition by GSK-2118436 is due to stabilization of the active conformation of B-raf by GSK-2118436, in contrast with stabilization of the inactive conformation of B-raf by sorafenib. Therefore, it may have the potential for overcoming generated resistance to inhibitors that bind to the inactive conformation of B-raf, as has been shown for other oncogene-dependent malignancies.

Clinical development

In a phase I trial of GSK-2118436, the drug was well tolerated, with limited toxicities. One patient reported dose-limiting toxicity of syncope (200 mg b.i.d.) and resumed reduced dosing. The most common adverse events were skin changes, low-grade cutaneous squamous cell cancer, headache, nausea, fatigue and vomiting. The MTD for GSK-2118436 was determined to be 150 mg p.o. b.i.d. (22).

A phase I/II study of GSK-2118436, was performed in patients with metastatic melanoma and other solid tumors. Sixty-one patients (52 with mutant B-raf melanoma) received 12-400 mg/day. Plasma concentrations were dose-proportional and exceeded the minimal therapeutic target at doses > 35 mg b.i.d. Concentration-dependent pERK inhibition was seen in tumors (maximum 93% inhibition) at GSK-2118436 concentrations of 140 ng/mL. In patients with mutant B-raf melanoma, an exposure-related decrease in FDG-PET metabolic uptake was noted, with 11 of 14 (79%) patients showing a decrease from baseline (range: –5 to –100%). In patients with mutant B-raf melanoma (without brain metastasis) 18 of 30 (60%) patients had a > 20% tumor decrease by RECIST at first restaging (8-9 wkeeks). Maximum tolerated dose had not yet been reached. Clinical activity with minimal toxicity was observed at multiple dose levels in mutant B-raf tumors (23).

RAF-265

Target specificity

RAF-265 is an orally active agent that selectively inhibits all Raf isoforms (A-Raf, B-raf, c-RAF), including *BRAF* V600E. It also inhibits VEGFR-2, c-Kit and PDGF-R-ß. It targets *BRAF* V600E and VEGFR-2 most potently in cell-based assays. RAF-265, previously known as CHIR-265 (Novartis), shows IC_{50} values of < 100 nM against B-raf mutant melanoma cell lines. RAF-265 has been shown to induce tumor regression and has antiangiogenic effects in animal models.

Clinical development

RAF-265 is currently in phase I clinical trials to determine the MTD, dose-limiting toxicities, the safety profile when given to patients with locally advanced or metastatic melanoma, and the plasma pharmacokinetics following oral administration, and to evaluate the potential pharmacodynamic effects of using tumor biopsies, peripheral blood samples and tumor imaging (24).

CONCLUSIONS

Raf kinase inhibitors have undoubtedly and permanently changed the landscape of metastatic therapy. Mutation-targeted approaches have already led to astounding results in early-phase trials that are on their way for confirmation in large randomized trials, with vemurafenib and GSK-2118436 leading the way. Particularly impressive is the early signal of efficacy of GSK-2118436 in active melanoma brain metastases. However, despite those impressive clinical successes, treatment-responsive patients ultimately relapse as a result of acquired resistance. Possibilities for the

development of acquired resistance include reestablishment of negative feedback and alternative activation of Ras/MEK/MAPK signaling (c-RAF bypass signaling), other *BRAF* mutations or amplifications, mutations in K-, N- or H-Ras, mutations in MEK-1, activation of alternative pathways that may drive proliferation and resistance to apoptosis (PI3K/Akt pathway activation), or upregulation of escape pathways (e.g., c-Met, Kit, FGFR and EGFR) through the activation of other receptor tyrosine kinases. It is critical to establish mechanisms by which resistance develops and to develop combination therapy or second-generation drugs to combat this resistance. Current research is focusing on defining a list of mutations linked to the effectiveness of specific targeted therapy; developing simple, high-throughput assays to detect point mutations and insertions/deletions, and then transferring all the information and technology to a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory utilized clinically for therapeutic decisions. This will allow us to update the panel with newly discovered mutations that may represent targets for future therapy. The future is to make this testing routine on all melanoma patients, allowing physicians to plan therapeutic approaches well in advance. Furthermore, these therapies will be brought into the early treatment of high-risk local or regional disease, where their adjuvant impact may be greatest.

DISCLOSURES

The authors state no conflicts of interest.

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