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MONOGRAPH

VEMURAFENIB

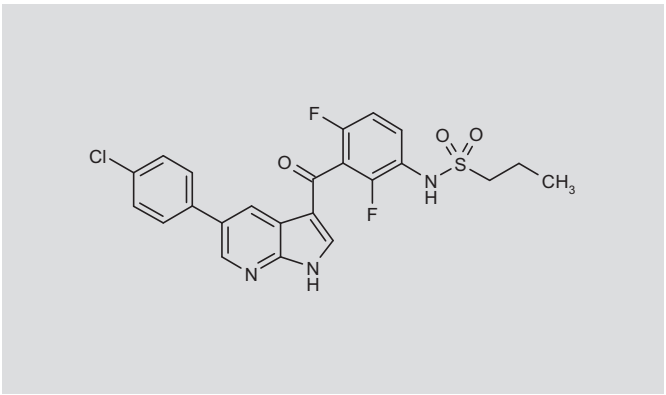
Prop INN; USAN

B-raf Kinase Inhibitor
Oncolytic

PLX-4032
R-7204
RG-7204
RO-5185426

N-[3-[5-(4-Chlorophenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-3-ylcarbonyl]-2,4-difluorophenyl]propane-1-sulfonamide

InChI: 1S/C23H18ClF2N3O3S/c1-2-9-33(31,32)29-19-8-7-18(25)20(21(19)26)22(30)17-12-28-23-16(17)10-14(11-27-23)13-3-5-15(24)6-4-13/h3-8,10-12,29H,2,9H2,1H3,(H,27,28)



$C_{23}H_{18}ClF_2N_3O_3S$

Mol wt: 489.922

CAS: 918504-65-1

EN: 423462

SUMMARY

Vemurafenib (RG-7204, PLX-4032) is a potent inhibitor of the V600E mutation-positive B-raf kinase. Mutations in this protein have been implicated in approximately 50% of melanomas, 30-70% of thyroid tumors, 30% of serous low-grade ovarian tumors and 10% of colorectal cancers. Vemurafenib has shown promising preclinical and clinical efficacy against mutant BRAF cell lines and tumors. Vemurafenib exhibits selectivity over a broad range of kinases, which has translated into cellular selectivity for cancer cell lines expressing BRAF^{V600E}, BRAF^{V600D} and BRAF^{V600R}, with no activity against cells lacking oncogenic B-raf. Pharmacokinetic analyses have shown that exposure increases with dose from 160 mg to 1,120 mg twice daily, and a dose of 960 mg twice daily was selected for phase II and III evaluation. Phase I and II clinical data have demonstrated promising activity, with the recently reported BRIM-2 study in patients with metastatic melanoma having met its primary endpoint, demonstrating a best overall response rate of > 50% in the context of manageable side effects.

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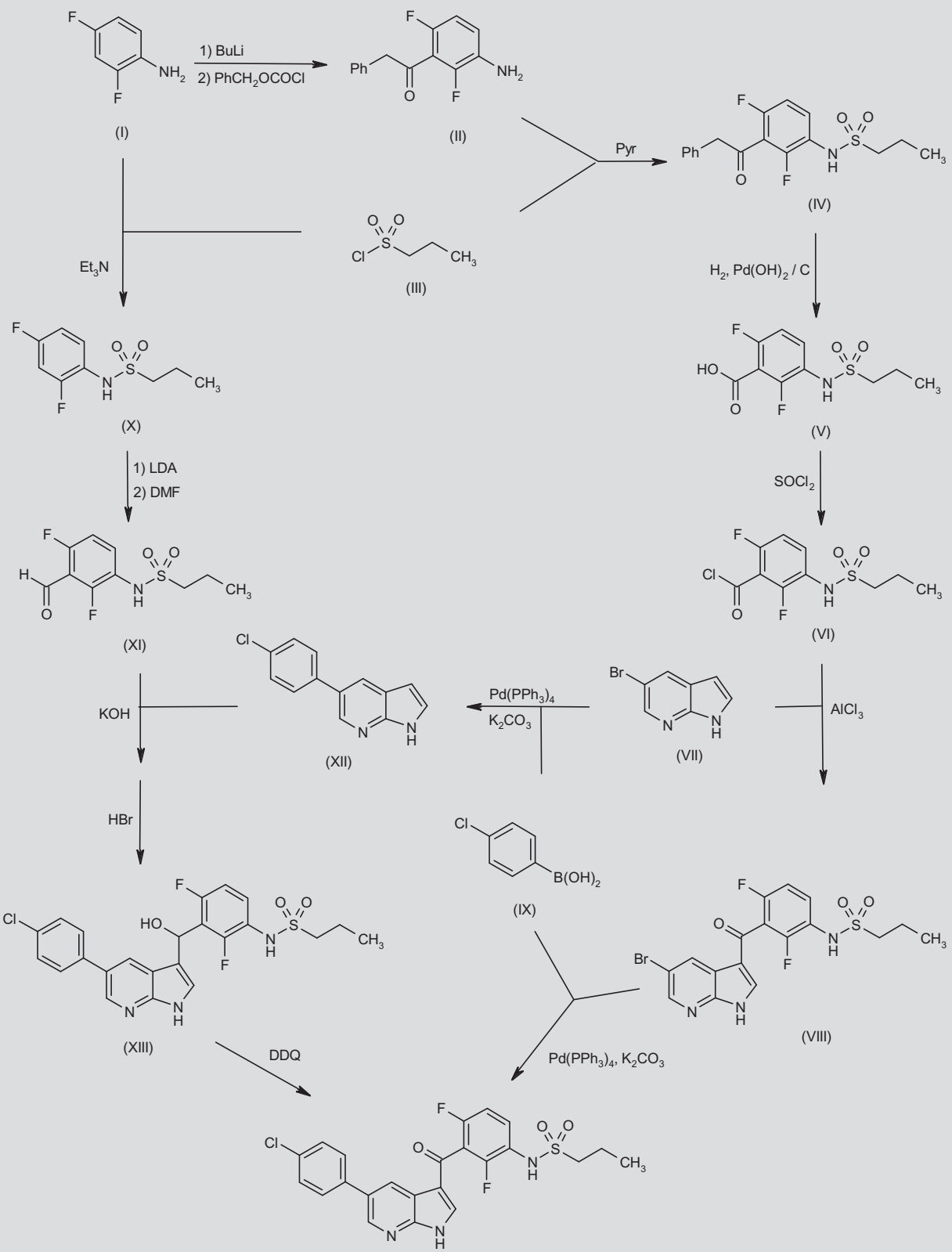
SYNTHESIS*

Vemurafenib can be prepared following two alternative strategies:

In one strategy, 2,4-difluoroaniline (I) is lithiated with BuLi in the presence of 1,2-bis(chlorodimethylsilyl)ethane in THF at –78 °C, and subsequently acylated with benzyl chloroformate, yielding benzyl 3-amino-2,6-difluorobenzoate (II). *N*-Sulfonylation of aniline (II) with propane-1-sulfonyl chloride (III) using pyridine in CH_2Cl_2 gives the corresponding sulfonamide (IV), which is then debenzylated with H_2 over $Pd(OH)_2$/C in MeOH to provide the benzoic acid derivative (V). After chlorination of acid (V) with $SOCl_2$ in refluxing toluene, the resulting acid chloride (VI) is subjected to Friedel–Crafts reaction with 5-bromopyrrolo[2,3-*b*]pyridine (VII) in the presence of $AlCl_3$ in CH_2Cl_2 to afford ketone (VIII). Finally, the aryl bromide (VIII) is submitted to a Suzuki coupling with 4-chlorophenylboronic acid (IX) in the presence of $Pd(PPh_3)_4$ and K_2CO_3 in acetonitrile (1, 2). Scheme 1.

In an alternative strategy, 2,4-difluoroaniline (I) is coupled with propane-1-sulfonyl chloride (III) by means of Et_3N in THF to give *N*-(2,4-difluorophenyl)propane-1-sulfonamide (X), which, after lithiation with LDA in THF, is formylated by reaction with DMF, yielding 2,6-difluoro-3-(propylsulfonamido)benzaldehyde (XI). Condensation of aldehyde (XI) with 5-(4-chlorophenyl)pyrrolo[2,3-*b*]pyridine (XII) (obtained by Suzuki coupling of 5-bromopyrrolo[2,3-*b*]pyridine (VII) with boronic acid (IX) in the presence of $Pd(PPh_3)_4$ and K_2CO_3 in acetonitrile/H_2O) using KOH in MeOH provides a mixture of the diarylcarbinol (XIII) and its corresponding methyl ether, which is further enriched in the desired alcohol (XIII) by demethylation with HBr in AcOH. Alcohol (XIII) is finally oxidized using DDQ in dioxane (1, 2). Scheme 1.

Scheme 1. Synthesis of Vemurafenib



BACKGROUND

B-raf is a key protein kinase component of the Ras/Raf pathway. Under normal physiological conditions, this critical intracellular signaling pathway relays extracellular signals to the nucleus that regulate gene expression (Fig. 1). The extracellular signals may be growth factors or hormones present in the microenvironment of the cancer cell, which bind to and activate cell surface receptor molecules. The activated receptor then, in turn, activates downstream components of the signaling pathway, perpetuating the signal through to the nucleus, where nuclear transcription factors regulate target gene transcription. By regulating the expression of target genes, the cell can respond to the extracellular environment in a variety of ways, including proliferation and survival via prevention of the cell's innate cell death mechanism –apoptosis (3, 4).

The B-raf protein is encoded by the *BRAF* gene. Approximately 8% of all human solid tumors are thought to harbor mutated B-raf and over 30 mutations in the *BRAF* gene have been associated with human cancers (5). The most commonly identified mutation in the *BRAF* gene arises in the kinase domain at nucleotide 1799, leading to a change in the V600 amino acid, resulting in constitutive activation of B-raf kinase. The mutated B-raf kinase is able to activate downstream components of the pathway even in the absence of an upstream (external) signal. This results in dysregulated downstream signaling, gene expression and, ultimately, excessive cell proliferation and survival (4-7). Oncogenic B-raf signaling is implicated in approximately 50% of melanomas, 30-70% of thyroid tumors, 30% of serous low-grade ovarian tumors and 10% of colorectal cancers (CRCs), and infrequently (< 5%) in a number of other cancers (4, 8-12). The pervasive nature of oncogenic B-raf signaling across human cancers has focused attention on the development of targeted anticancer agents able to attenuate the aberrant signaling generated by the mutant B-raf kinase.

Vemurafenib (RG-7204, PLX-4032) is a potent inhibitor of the V600E mutation-containing B-raf kinase that has shown promising preclinical activity and clinical efficacy (13-16). This agent is currently in clinical development for the treatment of human cancers harboring *BRAF* mutations, and here we review the preclinical pharmacology, mode of action, pharmacokinetics, safety and clinical efficacy.

PRECLINICAL PHARMACOLOGY

In vitro biochemical assays have shown that vemurafenib exhibits selectivity against a broad range of kinases. In panel testing of over

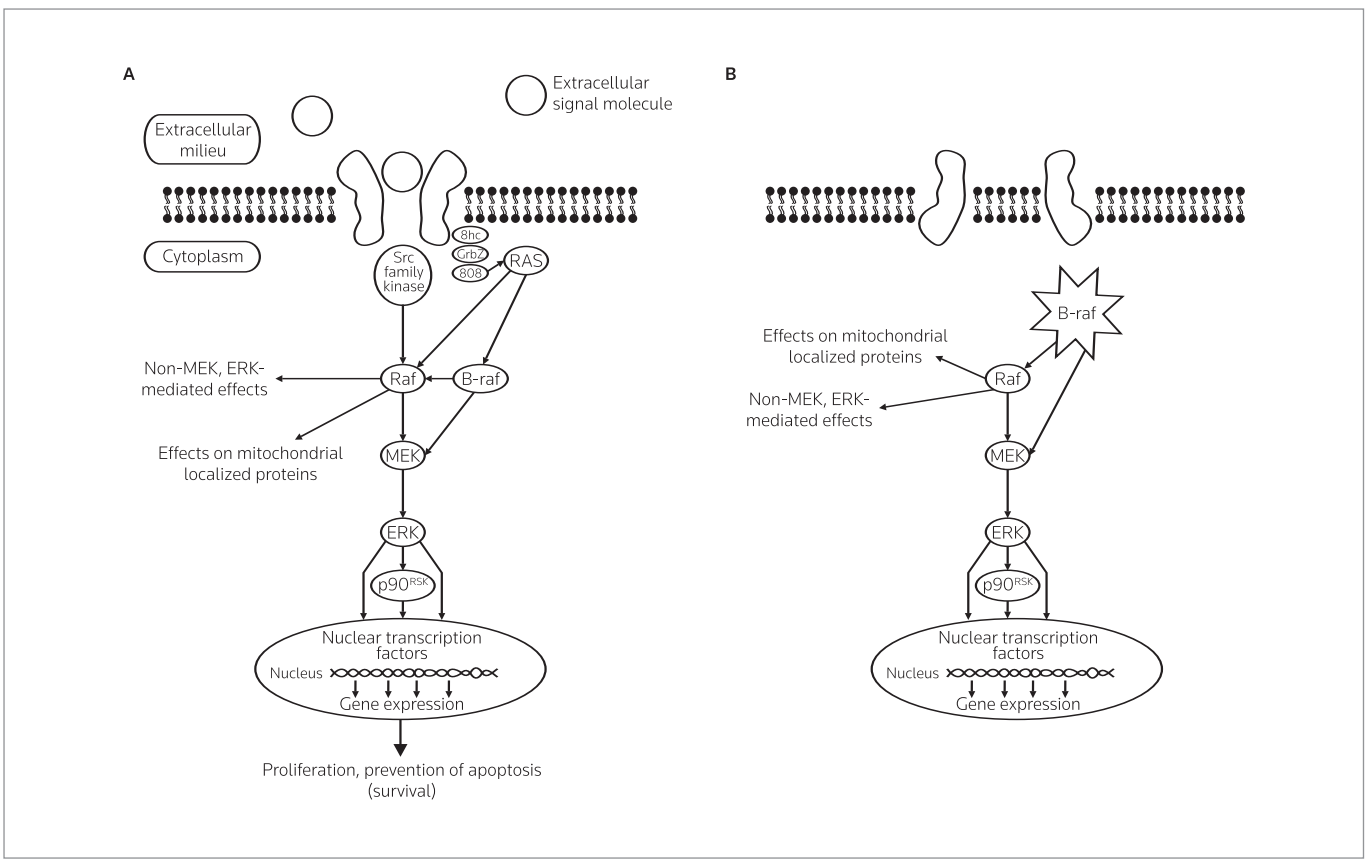


Figure 1. The Raf/MEK/ERK signaling pathway (panel A) and activation via constitutive B-raf activation (panel B). Adapted from McCubrey, J.A., Steelman, L.S., Chappell, W.H. et al. *Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance*. Biochim Biophys Acta 2007, 1773(8): 1263–84. Copyright © 2007, with permission from Elsevier.

200 kinases, vemurafenib showed similar potency for *BRAF*^{V600E} (31 nM; the most common mutation seen, encompassing 80% of *BRAF*-mutant melanoma tumors) and *RAF1* (48 nM), and selectivity versus other kinases (15). The vast majority of kinases were only minimally affected, with IC_{50} values of > 10 µM. However, several kinases (*BRAF*^{WT}, *RAF1*, *SRMS*, *ACK1*, *MAP4K5* and *FGR*) were reported to be inhibited at concentrations of ≤ 100 nM. In a separate biochemical assay, vemurafenib exhibited potent inhibitory activity against 10 other mutant B-raf kinases, including *BRAF*^{V600K}, *BRAF*^{V600D} and *BRAF*^{V600R}, with IC_{50} values ranging from 3 to 110 nM (personal communication, J. Tsai, Y. Ma and G. Habets).

The in vitro selectivity of vemurafenib has translated into cellular selectivity in a series of experiments designed to evaluate the effect of vemurafenib on Raf/MEK/ERK pathway inhibition and proliferation suppression in a panel of cancer cell lines (17). Cell lines tested for inhibition of MEK and ERK phosphorylation included melanoma cell lines expressing *BRAF*^{V600E}, *BRAF*^{V600D}, *BRAF*^{V600R} or *BRAF*^{WT}. Vemurafenib was reported to inhibit both phosphorylation of MEK and ERK, and cellular proliferation in all *BRAF*^{V600E}-expressing melanoma cell lines tested, including COLO 829 and LOX. Vemurafenib also exhibited potent inhibitory effects on MEK and ERK phosphorylation and cellular proliferation in melanoma cell lines that expressed other mutations at the V600 position, such as *BRAF*^{V600D}, *BRAF*^{V600R} and *BRAF*^{V600K}, but not in cells with wild-type *BRAF* (17-19). Vemurafenib lacked antiproliferative activity in cell lines expressing wild-type B-raf proteins, including those from melanomas and other tumor types such as lung, gastric, breast, pancreatic and skin tumors. Activity was reported in one additional breast cancer cell line which expressed *BRAF*^{V600E} and wild-type *RAS* (17).

Lee and coworkers also evaluated the in vitro activity of vemurafenib against a panel of melanoma cell lines with and without V600E-mutated *BRAF* genes and confirmed both concentration-dependent and V600E-dependent inhibition of pERK for up to 72 hours (20). Suppression of ERK and MEK phosphorylation by vemurafenib correlated with inhibition of cellular proliferation in melanoma cells harboring mutations at the V600 position. Thus, vemurafenib displays a high degree of selectivity against *BRAF*^{V600E} kinase in mechanistic and antiproliferative cellular assays.

The effect of three doses of vemurafenib (12.5, 25 and 75 mg/kg twice daily) on antitumor activity and survival was determined in vivo using the murine LOX melanoma xenograft model (17). Vemurafenib significantly inhibited tumor growth and induced tumor regression at all three doses studied (complete regression was reported in 10/10 mice treated with both 25 and 75 mg/kg twice daily, and in 5/9 mice treated with 12.5 mg/kg twice daily; 4/9 mice treated with 12.5 mg/kg twice daily showed partial tumor regression) (Fig. 2). Vemurafenib was also reported to significantly increase survival relative to vehicle in a dose-dependent manner. Eight of 10 mice in the 75 mg/kg twice daily group were considered to have been completely cured and died of natural causes. No gross signs of metastasis were observed at necropsy. However, tumors were reported to recur following complete regression in mice treated with lower doses. In the A-375 and COLO 829 melanoma models, vemurafenib 75 and 100 mg/kg twice daily was shown to induce potent antitumor activity and improve animal survival. In the A-375 model, survival was prolonged by 227% compared with vehicle-treated mice (75 mg/kg twice daily for 11 days; $P < 0.0001$). In the COLO 829 model, survival was prolonged by 61% compared with vehicle-treated mice (100 mg/kg for 21 days). In a separate analysis in the *BRAF*^{WT}-expressing C8161 melanoma model, tumors treated with vemurafenib 100 mg/kg twice daily for 12 days grew at similar rates to tumors treated with vehicle, with no tumor growth inhibition reported, while V600E-positive tumors were largely eradicated (20). These observations, together with the data generated in *BRAF*^{V600E}-expressing xenograft models, have shown the in vivo selectivity of vemurafenib for *BRAF*^{V600E}.

In vitro and in vivo studies have provided evidence for the mode of action of vemurafenib (15, 17, 18, 21-24). As a potent and selective inhibitor of mutant *BRAF*, vemurafenib was designed to suppress mitogen-activated protein kinase (MAPK) signaling via suppression of B-raf activity. The only known substrate for B-raf is mitogen-activated protein kinase kinase (MEK). Phosphorylation of MEK by B-raf results in activation of MEK; pMEK in turn phosphorylates ERK, and pERK translocates into the nucleus, where it activates transcriptional factors that are responsible for upregulating cell proliferation and survival (Fig. 1). The studies described above demonstrate that vemurafenib potently inhibits MEK phosphorylation and activation, which consequently inhibits ERK phosphorylation and ultimately cell proliferation in tumor cells expressing the mutant *BRAF* gene.

PHARMACOKINETICS AND METABOLISM

Exposure-dependent tumor response has been reported using a LOX *BRAF*^{V600E}-mutant melanoma xenograft model. Pharmacodynamic effects, determined by comparison of the percentage of pMEK/MEK and pERK/ERK inhibition in vemurafenib-treated tumors with vehicle-treated tumor samples suggested a correlation. In this case, the highest plasma concentration of vemurafenib corresponded to the highest mean percentage inhibition of MEK and ERK phosphorylation (17).

A total of 87 patients (including 81 with melanoma) were recruited to a phase I study and received doses of up to 1120 mg twice daily. Pharmacokinetic analyses in this study have shown that exposure (AUC) increased with dose from 160 mg to 1120 mg twice daily (Fig. 3A). Patients were exposed to relatively constant daily levels at steady state that were between six and nine times the mean level on day 1 at the dose of 960 mg twice daily (Fig. 3B). The mean half-life of vemurafenib was ~50 hours and the AUC for the dose of 960 mg twice daily was 1,741 ± 639 µM/hour (25). The dose of 960 mg twice daily was selected for phase II and III evaluation. Divided dosing was continued despite the long half-life, in order to avoid a potentially unacceptable number of capsules per dose. Phase I pharmacodynamic analyses showed that tumor levels of phosphorylated ERK, cyclin D1 and KI-67 were markedly reduced at day 15 compared with baseline (n = 7 patients treated with vemurafenib 960 mg twice daily), indicating that vemurafenib inhibited the MAPK pathway, resulting in decreased cell proliferation (15, 25). Additionally, there was a clear relationship between AUC, pharmacodynamic changes and clinical responses. Responses were largely seen only when

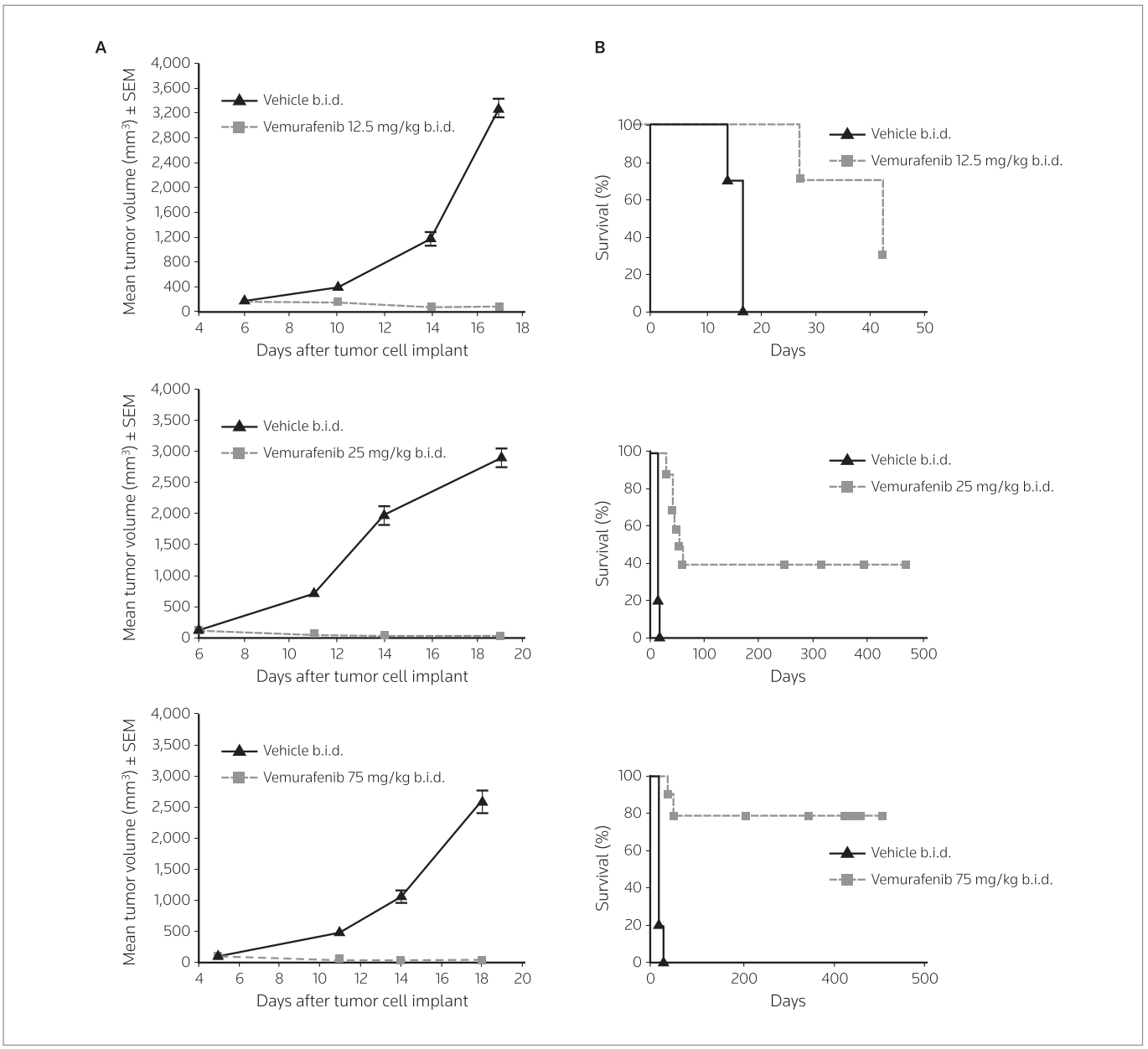


Figure 2. Murine tumor growth inhibition and survival in the *BRAF*^{V600E}-bearing LOX melanoma xenograft model. Adapted and reprinted by permission from the American Association for Cancer Research: Yang, H., Higgins, B., Kolinsky, K., et al. *RG7204 (PLX4032), a selective BRAF*^{V600E} *inhibitor, displays potent anti-tumor activity in preclinical melanoma models*. Cancer Res 2010, 70(13): 5518-27.

patients demonstrated over 90% phosphorylated ERK inhibition intratumorally (Fig. 4).

Several further pharmacokinetic studies are under way to provide more in-depth information on the pharmacokinetics, the potential interaction with cytochrome P450 enzymes, and the distribution and metabolism of vemurafenib. Results from these studies are expected in 2011. Another study to evaluate the effect of food on the pharmacokinetics of vemurafenib has also been initiated.

SAFETY

Preclinical animal safety studies revealed no safety signals at daily doses of up to 1000 mg/kg/day, and no adverse effects were detected in a standard battery of safety pharmacology studies (15). This was despite very high exposure levels of 2,600 µM/hour and 820 µM/hour for up to 26 and 13 weeks, respectively, in rats. No histological changes were observed in the skin of any animal at any dose or duration.

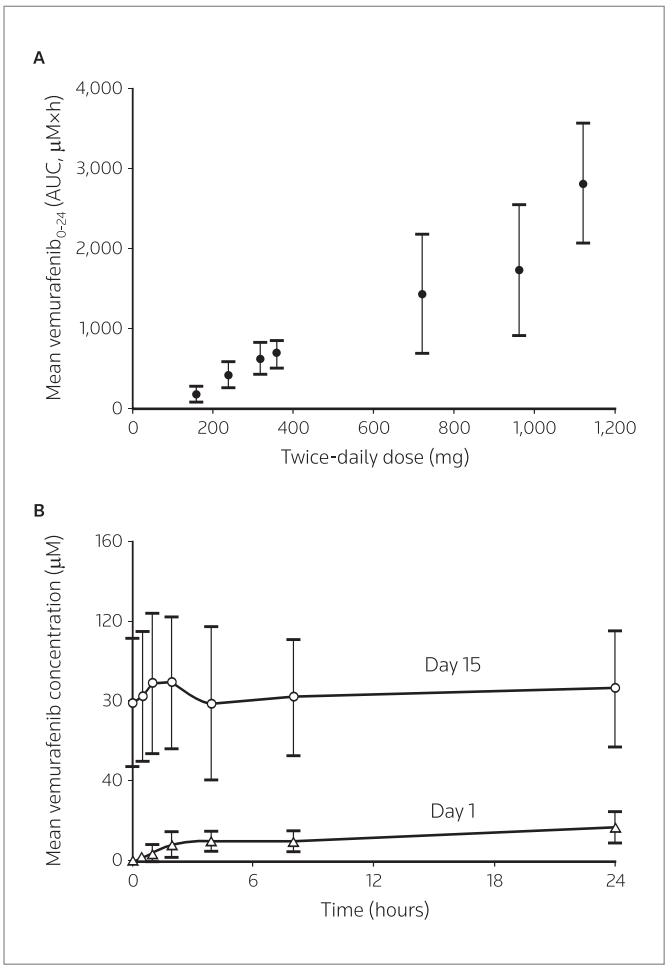


Figure 3. Pharmacokinetic profile of vemurafenib in humans. Dose-dependent exposure (panel A) and relatively constant daily exposure at steady state (panel B). Published with permission from Flaherty, K.T., Puzanov, I., Kim, K.B., et al. *Inhibition of mutated, activated BRAF in metastatic melanoma*. N Engl J Med 2010, 363(9): 809-19. Copyright © 2010, with permission from New England Journal of Medicine.

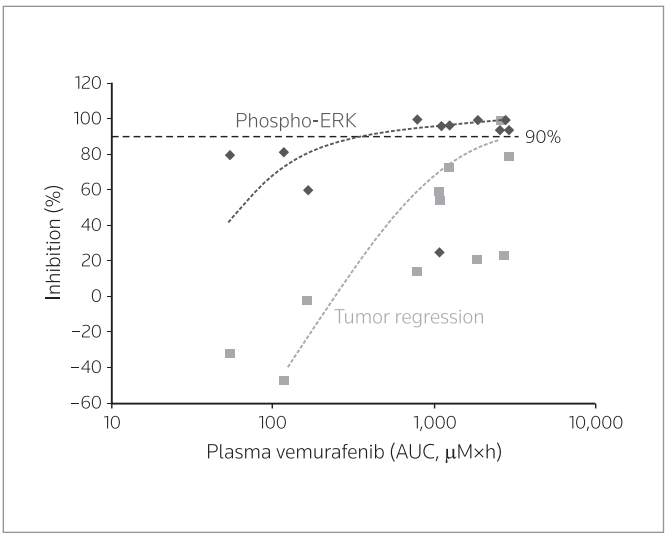


Figure 4. Melanoma regressions correlate with > 90% phosphorylated ERK inhibition.

In the preclinical pharmacology studies reported by Yang and coworkers, the treatment described above with vemurafenib was well tolerated. Mean values for weight loss were < 5% in all treated dose groups, and no treatment-related mortality was reported in any dose group. No clinical signs of toxicity were reported at any time during the studies (17).

The phase I trial reported by Flaherty and coworkers and described above consisted of a dose-escalation phase followed by an extension phase at the recommended phase II dose of 960 mg twice daily (25). The most frequent adverse events (AEs) were arthralgia, rash, nausea, photosensitivity, fatigue, pruritus and palmar–plantar dysesthesia. Most events were mild to moderate in severity. Cutaneous squamous cell carcinoma occurred at a rate of 31% (10/32 patients) during the extension phase of the study. The median time to occurrence of these lesions was 8 weeks; the majority were resected and none led to discontinuation of treatment. The majority of excised lesions reviewed by a central pathology laboratory were classified as squamous cell carcinoma, keratoacanthoma subtype, with a low likelihood of invasive or metastatic potential (26).

CLINICAL STUDIES

The dose-escalation portion of the phase I study reported by Flaherty and coworkers and described above was followed by an extension phase during which patients received the recommended phase II dose of 960 mg twice daily until disease progression (25). The two extension cohorts consisted of 32 patients with melanoma (25) and 21 patients with CRC (27). Among the 32 patients with melanoma carrying a *BRAF*^{V600E} mutation who took part in the extension phase, a total of 26 patients (81%) achieved an objective response, with 2 patients achieving a complete response (Fig. 5) (25). Data on fluorodeoxyglucose-positron emission tomography (FDG-PET) responses are available for 19 of these patients and showed that FDG-PET is a useful marker for early biological response (28). Among patients with metastatic disease, responses were recorded at all metastatic sites and there was an improvement in symptoms for those with symptomatic disease, including a reduction in the requirement for narcotic pain relief within 1-2 weeks (although it should be noted that these data were anecdotal and were not systematically collected). Evaluation of patients in this extension arm is ongoing; a confirmed response rate of 59% and a progression-free survival (PFS) of 7.61 months were recorded as of September 30, 2010.

In the CRC extension cohort, 19 of the 21 patients were evaluable for efficacy at the time of latest reporting. Again, all patients received vemurafenib 960 mg twice daily. One patient achieved a partial response and four patients achieved minor responses (≥ 10% shrinkage). The median PFS was 3.7 months, with two patients still on study at the time of the initial data presentation (27).

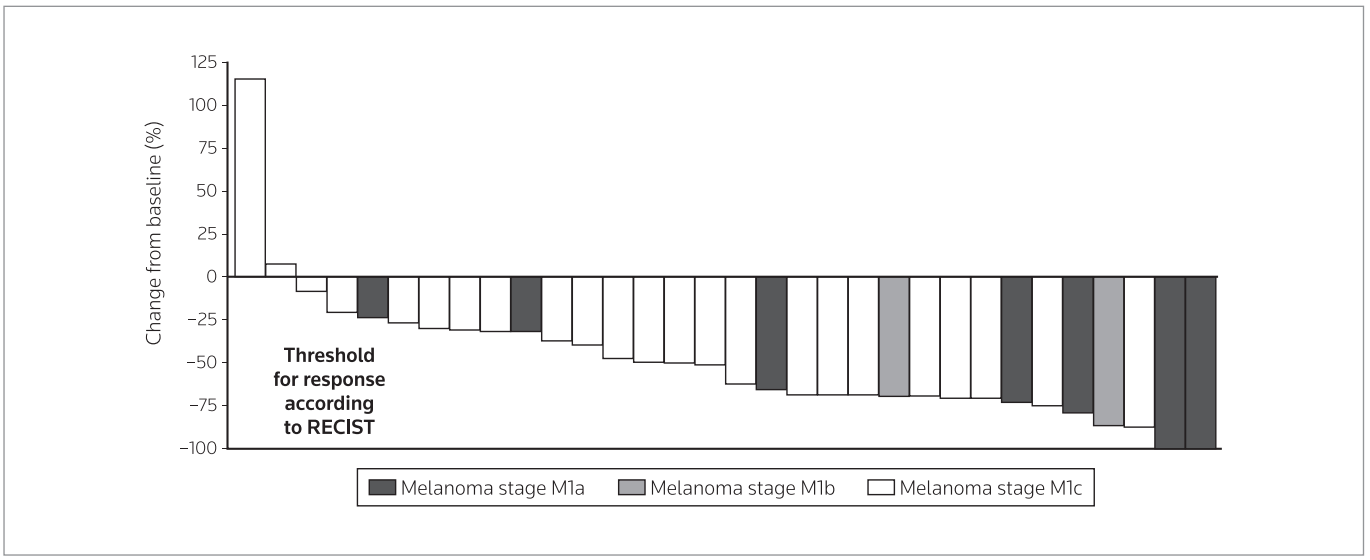


Figure 5. Best overall response among 32 patients with melanoma treated with vemurafenib 960 mg twice daily. Published with permission from Flaherty, K.T., Puzanov, I., Kim, K.B., et al. *Inhibition of mutated, activated BRAF in metastatic melanoma*. N Engl J Med 2010, 363(9): 809-19. Copyright © 2010, with permission from New England Journal of Medicine.

Three patients with thyroid cancer took part in the dose-escalation phase of the phase I study; 1 patient achieved a partial response of 11 months' duration, and the remaining 2 patients achieved stable disease of 12 and 13 months' duration (25).

A phase II (BRIM 2) study of vemurafenib in patients with metastatic melanoma was recently reported (29). In all, 344 patients were screened, 132 of whom were eligible for treatment. The rate of *BRAF*^{V600E} mutation in the screened population (N = 344) was 56%. Preliminary best overall response rate (BORR; primary study endpoint) was > 50% as assessed by an independent review committee (IRC). The median response duration was nearly 7 months and median PFS was > 6 months for the entire cohort. Side effects (AEs, serious AEs, dose reductions) were manageable, the most common being arthralgia, rash, photosensitivity, fatigue and alopecia. As previously reported in the phase I/II trial, squamous cell carcinoma primarily of keratoacanthoma type was reported in 32 patients, representing about 25% of patients. Thus, BRIM-2 met its primary endpoint, demonstrating a BORR (IRC) whose lower limit of 95% confidence interval was > 40. As of the clinical cut-off date of September 27, 2010, 50 patients remained on protocol. The full results will be reported at a later date.

A number of additional studies are currently ongoing, including phase I studies to further characterize the pharmacokinetics of vemurafenib (NCT01164891, NCT01107418, NCT01001299). A phase III study (BRIM3; NCT01006980) is also under way to compare the efficacy of vemurafenib with that of dacarbazine in patients with previously untreated metastatic melanoma. Enrollment for this study has been completed and primary outcome data (co-primary endpoints of overall survival and PFS) are expected in 2011.

Paradoxical activation of ERK by Raf inhibitors has been reported in cells that lack a *BRAF* mutation. Three recent reports have explored the potential mechanism(s) for this activation by showing that selective B-raf inhibitors, such as PLX-4720 (an analogue of vemurafenib), 885-A and GDC-0879 (selective B-raf inhibitors of different chemical series), stimulate MEK/ERK signaling via c-RAF activation in the presence of an upstream activator (e.g., activated receptor tyrosine kinase, *RAS* mutation) in melanoma and other cell lines lacking *BRAF* mutations (30-32). These studies support a model in which B-raf-specific inhibitors induce Ras/GTP-dependent c-RAF activation via the formation of B-raf–c-RAF heterodimers or c-RAF homodimers, followed by recruitment of c-RAF to the plasma membrane, triggering activation of the MEK/ERK pathway. These reports emphasize the importance of selecting patients with *BRAF*^{V600E} mutations for treatment with vemurafenib. The cobas® 4800 *BRAF*^{V600} mutation assay is a companion PCR-based diagnostic test that is designed to detect the *BRAF*^{V600E} mutation (1799T>A), the most common *BRAF* mutation in melanoma. It is being developed by Roche Molecular Systems, Inc. (Pleasanton, CA, USA) as a clinically validated companion diagnostic test to identify patients who are candidates for treatment with vemurafenib. This test is currently being used to identify patients for clinical trials evaluating vemurafenib treatment.

Initial investigations into potential mechanisms of acquired resistance suggest that there are several genetic and signal transduction alterations that can circumvent B-raf inhibition. The number of cell lines that have been treated chronically with vemurafenib to generate acquired resistance is small, and the number of tumor samples acquired at the time of clinical disease progression and thoroughly characterized is even smaller. Nonetheless, a few key observations have been reported recently that support reactivation of the MAP kinase pathway or Ras effector pathways as resistance mechanisms for which there is support from both in vitro and ex vivo analyses. These observations follow a series of reports that highlight the par-

adoxical effect of Raf inhibitors to activate the MAPK pathway, as noted above (30-32). This effect is primed by upstream events such as *RAS* mutation, believed to be due to the asymmetric behavior of Raf dimers. Binding of inhibitors to one Raf promoter appears to result in allosteric enhancement of the catalytic activity of the neighboring, inhibitor-free promoter. *RAS* mutation would additionally stimulate alternative downstream pathways. In one case, the appearance of inactivating *NRAS* mutation coexisting with the *BRAF*^{V600E} mutation at the time of disease progression has been documented. In other cases, a marked increase in the expression of beta-type platelet-derived growth factor receptor (PDGF-R-β) has been observed and appears to mediate upregulation of phosphatidylinositol 3-kinase (PI3K) pathway signaling (33). Recently, Villanueva and coworkers reported that a combination of flexible Raf isoform switching and enhanced insulin-like growth factor receptor I (IGF-I receptor)/PI3K signaling was involved in mediating resistance to the B-raf inhibitors (34). This could be overcome by combined IGF-I receptor and MEK inhibition (35, 36). Loss of the phosphatase and tensin homolog (PTEN, a negative regulator of the PI3K/Akt signaling pathway) was also found in a relapsed patient sample, further suggesting that combined inhibition of the MAPK and PI3K pathways could improve tumor control (34). Lastly, signaling through c-Cot/TPL-2, which has previously been described as an activator of MEK signaling, can promote resistance in previously sensitive cell lines, and increased expression of this molecule in tumor samples derived from patients progressing following initial response supports the potential relevance in vivo (37). In another study examining upregulation of Raf isoforms, Akt activation and acquisition of *KRAS* mutation were observed in an in vitro vemurafenib-acquired resistance model, implying that multiple mechanisms leading to increased pathway activity are at play (Su, F. et al., manuscript submitted 2011). Importantly, in all of these analyses, acquired secondary mutations in B-raf have not been observed, suggesting that compensatory signaling to other molecules is the primary mode of resistance.

SOURCES

Plexxikon, Inc. (US); codeveloped with Roche and Genentech (a member of the Roche Group).

DISCLOSURES AND ACKNOWLEDGMENTS

IG, JS and KF have acted as consultants for Roche Pharmaceuticals. JG, FS and RL are employees of Roche Pharmaceuticals. KN and GB are employees of Plexxikon, Inc. Support for third-party writing assistance for this manuscript, furnished by Tracey Lonergan, PhD, was provided by Roche Pharmaceuticals.

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