Vemurafenib: A First in Class Serine-Threonine Protein Kinase Inhibitor for the Treatment of Malignant Melanoma With Activating BRAF Mutations

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Vemurafenib (ZELBORAF, Hoffmann-La Roche Inc; Figure 1) is a first-in-class selective inhibitor of the BRAF serine-threonine kinase, FDA approved for the treatment of metastatic melanoma harboring activating BRAF mutations in August, 2011.

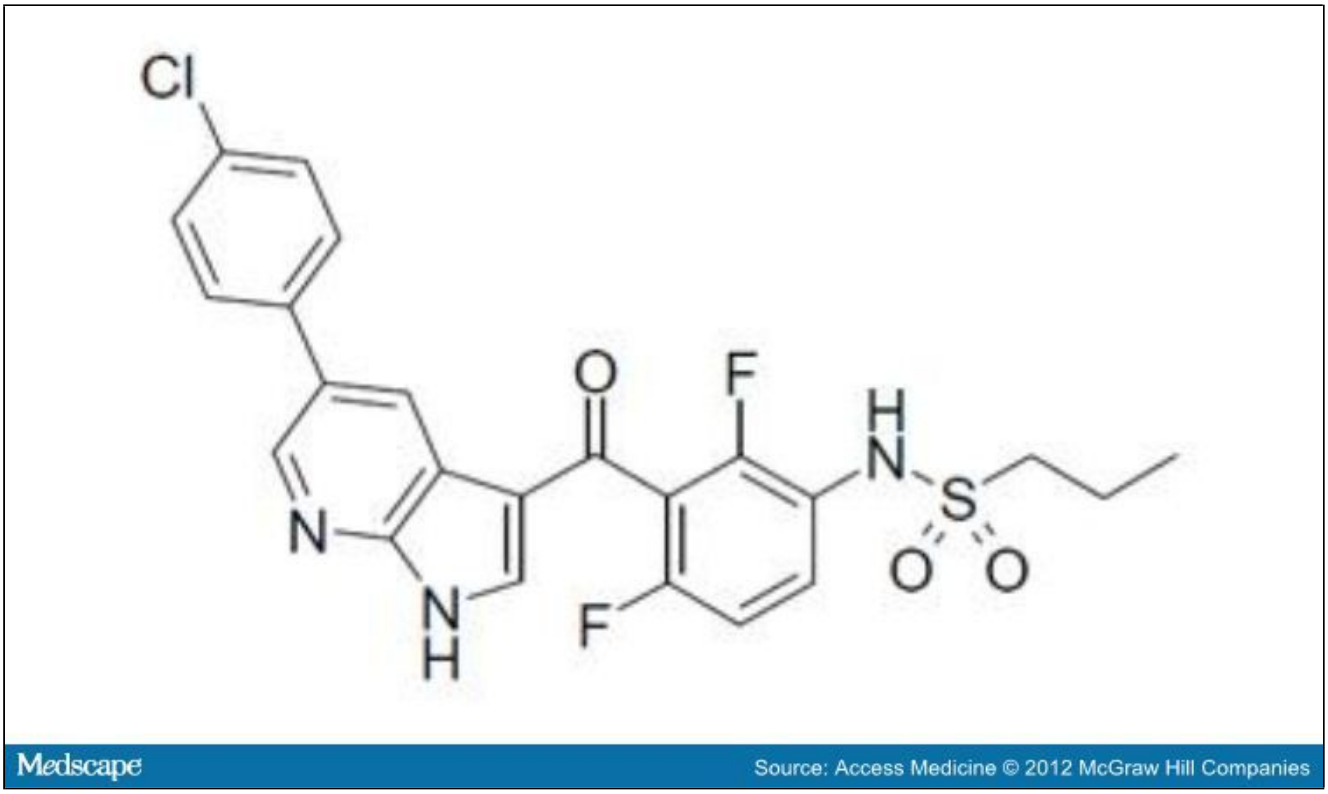

Figure 1.

Chemical structure of vemurafenib (chemical name propane-1-sulfonic acid {3-[5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridine-3-carbonyl]-2,4-difluoro-phenyl}-amide; also known as PLX4032, RG7204, RO5185426, 1029872-54-5; PubChem SID 131480743)

BRAF is a component of the MAP kinase pathway, signaling in parallel with ARAF and CRAF, downstream of RAS GTPases[1] (Figure 2). MEK is the best described substrate of BRAF. In 2002, a focused genetic analysis of components of the MAP kinase pathway revealed activating mutations in BRAF in 7-8% of all cancers.[2] BRAF mutations are found most commonly in melanoma (50%), papillary thyroid cancer (40%), cholangiocarcinoma (15%), colorectal cancer (8%), and non-small cell lung cancer (3%). The vast majority of BRAF mutations confer constitutive activation of the kinase such that the kinase activity is several hundred-fold higher than wild-type BRAF. This contributes to RAS-independent cellular proliferation and clonogenic growth. Other BRAF mutations have been described as oncogenic, but do not appear to exert that effect through MEK phosphorylation.[3] The therapeutic role of vemurafenib has only been established in cancers harboring activating mutations which result in substitution of other amino acids for the valine residue at the 600 position in the BRAF amino acid sequence. The most of common of those, accounting for 90% of all BRAF mutations, is the mutation that results in a substitution of glutamate for valine (V600E; Figure 2).

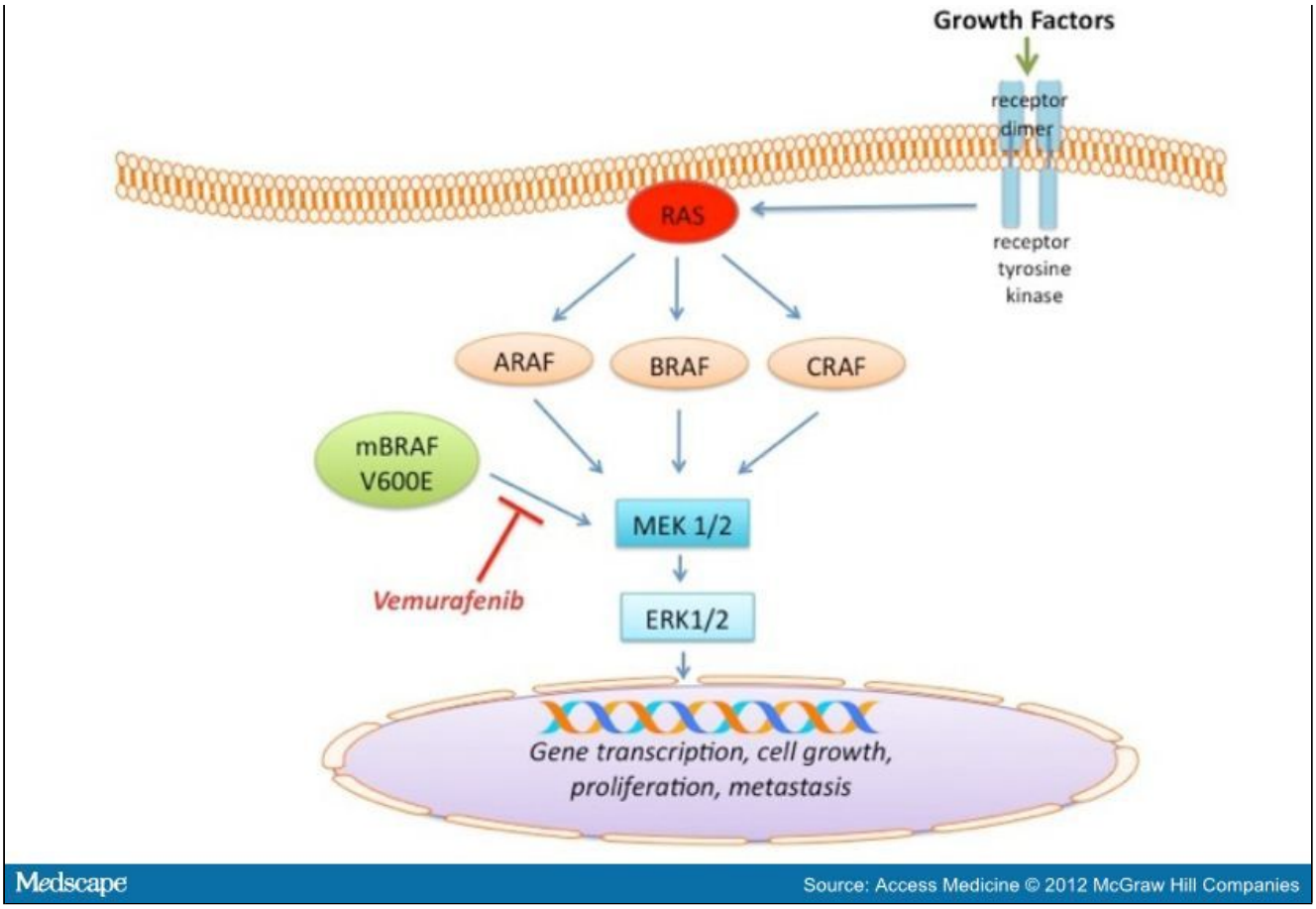

Figure 2.

Schematic representation of the mechanism of action of vemurafenib in cancers harboring activating BRAF (V600E) mutations.

Vemurafenib is a reversible, ATP-competitive inhibitor of the kinase domain of BRAF.[4] Owing to a high degree of homology in this domain between BRAF and CRAF and a small number of additional kinases, the drug has shown inhibitory activity against CRAF, ARAF, SRMS, ACK1, MAP4K5, and FGR *in vitro* using purified kinases. However, in intact cells, vemurafenib only blocks MEK activation in cells that harbor the activating BRAF mutations. In BRAF wild-type cells, vemurafenib paradoxically increases MEK activation by stimulating the kinase activity of BRAF dimers.[5-7] In the setting of activating mutations, BRAF can phosphorylate MEK as a monomer and its activity inhibited as the concentration of vemurafenib is increased. This differential effect on the signaling activity in the MAP kinase pathway is thought to contribute to the therapeutic index of vemurafenib. Only cancer cells that have activating BRAF mutations are growth-inhibited or undergo cell death upon vemurafenib exposure.[4] However, increased MEK activation in normal cells appears to underlie some of the toxicities observed with vemurafenib treatment in patients.[8]

In clinical trials, increasing the dose of vemurafenib results in proportional increases in exposure.[9] The maximum tolerated dose is 960 mg orally twice daily. At this dose, maximum concentration at steady state is approximately 86 ± 32 mM and AUC (0-24 hr) is 1741 ± 639 mM*hr. These concentrations are much higher than those needed for growth inhibition of BRAF mutant melanoma *in vitro*.[4] However, vemurafenib is more than 99% protein bound and the precise amount of free vemurafenib that diffuses into cells and engages targeted kinases *in vivo* is not known.[10] As a consequence of this high protein binding, the volume of distribution is relatively high and variable (approximately 100 L with 66% inter-patient variability). Clearance is approximately 30 L/day. The mean $t_{1/2}$ is 50 hrs, resulting in 6-9 fold accumulation between day 1 and day 15. Twice-daily dosing is recommended in order to improve tolerability, given that four large 240 mg tablets are taken at each dose.

Vemurafenib is metabolized by cytochrome P450 isoenzymes (predominantly CYP3A4) into inactive metabolites.[10] The concomitant use of strong inducers or inhibitors of CYP3A4 is not advised. The vast majority of vemurafenib is eliminated in the feces. Vemurafenib is neither a strong inducer nor inhibitor of CYP450 isoenzymes, and is not anticipated to significantly alter the metabolism of other co-administered medications.

At doses of 240 mg orally twice daily and higher, tumor regression was observed in patients with V600E BRAF metastatic melanoma.[9] Tumor biopsies performed before and after two weeks of therapy demonstrate greater than 80% inhibition of the MAP kinase pathway activity and consistent decreases in tumor uptake of radiolabelled glucose.[4]

Efficacy has been documented only for vemurafenib monotherapy and in patients with metastatic melanoma harboring V600 BRAF mutations. Approximately 90% of patients experience some degree of tumor regression early in the course of therapy.[9,11] Fifty to 60% of patients have a sufficient magnitude of tumor regression to constitute a partial or complete response by the conventional metrics used in cancer clinical trials. With continued treatment, the emergence of resistance can be seen as soon as 6-8 weeks following initial documentation of response. However, a subset of patients maintains response beyond 18 months.[12] The median duration of response is 8 months. In a disease as aggressive as metastatic melanoma it is not surprising that vemurafenib has demonstrated a survival advantage compared to historically minimally effective single-agent chemotherapy. Compared to dacarbazine, vemurafenib reduced the risk of death by 63% ($p < 0.001$).[11] Long-term follow-up of previously treated clinical trial cohorts suggests that the median overall survival is 14 to 16 months, significantly improved over the historically observed median survival of 6 to 10 months.[13]

Understanding mechanisms of resistance has been the focus of intense investigation with the hope of uncovering molecular mechanisms that can be directly targeted in future vemurafenib-based combination regimens. Most commonly, MEK activation is

restored at the time of disease progression following initial response.[14] There appear to be several mechanisms by which this reactivation can occur and combined targeting of BRAF and MEK is a strategy that is being actively investigated in clinical trials to suppress these mechanisms.[15,16] In other cases, disease progression is associated with persistant inhibition of the MAP kinase pathway and signaling through the parallel PI3 kinase pathway has been implicated.[17] Thus, it is possible that different subsets of BRAF mutant cancers will require different drug combination regimens to overcome resistance. Additional evidence suggests that BRAF inhibition has positive consequences with regard to immunologic recognition of melanoma cells, and may support investigation of combination regimens with immunotherapies.[18]

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