## Title
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# Targeted Therapy for Melanoma

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**Abstract**

Vemurafenib and dabrafenib, two potent tyrosine kinase inhibitors (TKI) of the $BRAF^{V600E}$ kinase, are highly effective in the treatment of $BRAF^{V600}$ mutant metastatic melanoma. These are selective type I inhibitors (functional against the active conformation of the kinase) of the RAF kinases, which is a key player in the mitogen activated protein-kinase (MAPK) pathway. $BRAF^{V600}$ mutations are present in approximately 7% of all cancers, including high frequencies of mutations reported in 50% of advanced melanomas and 100% of hairy cell leukemias. As with most targeted therapies, resistance to BRAF inhibitors is an issue, and mechanisms of resistance are varied. Combining BRAF inhibitors with MEK inhibitors such as trametinib delays the development of resistance. Rationally combining targeted therapies to address the mechanism of resistance or combining BRAF inhibitors with other effective therapies such as immunotherapy may result in further improvement in outcomes for patients.

**List of Keywords**: melanoma, BRAF, vemurafenib, dabrafenib, MEK, trametinib

**Current Treatment Options for Advanced or Metastatic Melanoma**

Until recently, there was a dearth of effective treatments for surgically unresectable or metastatic melanoma. At best, cytotoxic chemotherapy such as dacarbazine yields a response rate of approximately ten percent. Similar response rates are seen with immunotherapies, such as interleukin-2 (IL-2), but these responses may be extremely durable. Neither chemotherapy nor IL-2 clearly results in improved overall survival (OS), however .

The outlook for patients with advanced melanoma significantly brightened with the identification of specific BRAF and MEK inhibitors and immune modulating antibodies as effective therapies for this disease. Ipilimumab, a cytotoxic T-lymphocyte antigen (CTLA4) blocking antibody, was approved for treatment of metastatic melanoma. Responses to ipilimumab are on the order of 10-15%. Unlike the afore-mentioned agents, ipilimumab does improve median OS compared to the control arms in randomized clinical trials . In September 2014, the programmed death-1 (PD-1) blocking antibody pembrolizumab (MK-3475, Merck) was FDA-approved for metastatic melanoma that has progressed on ipilimumab and BRAF inhibitors (if *BRAF* mutated). Pembrolizumab has an overall response rate (ORR) of 24% with many of these responses ongoing for six months or longer . In all, there have been 6 FDA-approved therapies for the treatment of patients with metastatic melanoma since 2011.

High response rates for $BRAF^{V600}$ mutant metastatic melanoma are seen with as the type 1 BRAF inhibitors vemurafenib (formerly PLX4032) and dabrafenib (formerly GSK2118436) . Unfortunately, though initial responses to these agents are impressive, progression free (PFS) is on the order of 6-7 months. Combining BRAF inhibition with MEK inhibition results in improved PFS compared to BRAF inhibition alone . However, not all melanomas express the mutated BRAF protein, and not all melanomas with mutant *BRAF* are responsive to these targeted

therapies. Thus, effective therapies that address both *de novo* and acquired resistance to BRAF and MEK inhibitors remain a subject of active research. Understanding the biology of melanoma will be key in identifying strategies to address resistance to therapy.

**Targeting the MAPK pathway in melanoma**

BRAF is a serine-threonine protein kinase belonging to the RAF family of kinases, which is part of the MAPK signaling pathway. Under normal signaling conditions, binding of a growth factor to a RTK such as c-KIT activates RAS which then activates the RAF kinases. There are 3 identified RAF kinases: ARAF, BRAF and CRAF. RAF activation in turn phosphorylates MEK, leading to activation of ERK and subsequent phosphorylation of various targets that result in cell proliferation and other key biologic processes (Fig. 1). Dysregulation of the MAPK pathway is a key feature in the majority of melanomas. Indeed, about 20% of melanomas contain activating mutations in *NRAS* . Mutations in *KIT* and *KRAS* have also been identified. Approximately 50% of all melanomas contain a mutation in the *BRAF* gene, most commonly resulting in substitution of glutamic acid for valine at position 600 (V600E) . The *BRAF* $^{V600E}$ substitution leads to constitutive activation of this kinase and consequently, constitutive ERK signaling. Mutations in *BRAF* are, in general, mutually exclusive with other mutations of other proteins in the MAPK pathway , though recently, exceptions have been reported .

Inhibitors of RAF include type I inhibitors which selectively inhibit the activated RAF kinase, and type II inhibitors which inhibit the resting RAF. Type II inhibitors like sorafenib do not have potent activity in *BRAF*$^{V600E}$ mutated cancers . In contrast, two clinically relevant type I TKIs that target BRAF$^{V600E}$ are vemurafenib and dabrafenib. As published in phase I, II, and III studies of vemurafenib, in patients whose tumors bear the BRAF$^{V600E}$ mutation, treatment of patients with

*BRAF*$^{V600E}$ advanced melanoma with vemurafenib resulted in response rates exceeding 50% by RECIST criteria, some degree of tumor response in over 80% of patients, resulting in improvements in PFS and OS . Data from the BRIM-3 phase 3 trial of vemurafenib demonstrated that treatment with vemurafenib confers an overall response rate of 48% compared to 5% in dacarbazine, the only FDA-approved chemotherapy for metastatic melanoma . The duration of response ranged from 2 to >18 months, with a mean duration of response of 6.7 months , though there are a few patients who have ongoing durable responses. Estimated OS at 6-months was 84% for vemurafenib, compared to 64% for dacarbazine. In the published updated analysis of BRIM-3, median OS was significantly longer in the vemurafenib group than in the dacarbazine group (13·6 months [95% CI 12·0—15·2] *vs* 9·7 months [7·9—12·8]; hazard ratio [HR] 0·70 [95% CI 0·57—0·87]; p=0·0008). Similarly, median PFS was improved (6·9 months [95% CI 6·1—7·0] *vs* 1·6 months [1·6—2·1]; HR 0·38 [95% CI 0·32—0·46]; p<0·0001) .

The phase I study of dabrafenib demonstrated 18/30 (60%) of patients demonstrated a > 20% tumor decrease by RECIST at first restaging . Similarly, the open label Phase II study of dabrafenib for BRAF$^{V600E/K}$ mutant melanoma, BREAK-2, 45/76 (59%, 95% CI 48.2 -70.3) with 7% complete responses. 13% of patients with *BRAF*$^{V600K}$ mutant melanoma had a confirmed response. For those with *BRAF*$^{V600E}$, median PFS was 6.3 months and median OS was 13.1 months, while it was 4.5 months and 12.9 months, respectively, for those with *BRAF*$^{V600K}$ . Furthermore, BREAK-3, the phase III study comparing dabrafenib to dacarbazine for first-line treatment of advanced melanoma randomized 250 patients 3:1 to receive either dabrafenib or dacarbazine. The primary end point was investigator-assessed PFS. Consistent with the Phase I data, the response rate was 52%, (95% CI 45-59) for the dabrafenib arm and 17% (95% CI 9-29) for the dacarbazine arm. There was a 3% complete response rate among patients who received dabrafenib. The median PFS was 5·1 months for dabrafenib and 2·7

months for dacarbazine, with a hazard ratio (HR) of 0·30 (95% CI 0·18-0·51; p<0·0001) . Given these positive data for BRAF inhibitors, vemurafenib received FDA approval in August 2011 for the treatment of patients with advanced or metastatic $BRAF^{V600E}$ mutant melanoma and dabrafenib was FDA-approved in May 2013.

Both vemurafenib and dabrafenib are generally well-tolerated. With vemurafenib, adverse events were mainly grade 2 or 3 in severity and included 18% incidence of cutaneous events (squamous cell carcinoma, keratoacanthoma, or both) managed by excision, arthralgia (21%), fatigue (13%), and 12% incidence of photosensitivity skin reactions, the most severe of which could be prevented by the use of sunblock. Adverse reactions requiring dose modifications or interruptions occurred in 38% of patients . For dabrafenib, adverse events reported in the phase I study included skin changes, low grade cutaneous SCC, headache, nausea, fatigue, and vomiting . In BREAK-2, rates of the most common AEs were: arthralgia (33%), hyperkeratosis (27%), and pyrexia (24%). Twenty-five patients (27%) had a serious AE and nine (10%) had squamous cell carcinoma. In the Phase III study, 53% of patients developed adverse events compared to 44% in the dacarbazine arm. These include hyperkeratosis, palmar-plantar erythrodysesthesia syndrome, headache, pyrexia, arthralgia, papilloma, and alopecia. Grade 3 or 4 adverse events were uncommon in either group .

**Limitations of BRAF Inhibitors**

Only melanoma cells with mutated *BRAF* are susceptible to inhibition by type I Raf inhibitors. This is hypothesized to be because mutant *BRAF* is locked in an activated conformational state, which selectively allows inhibitor binding at lower concentrations than needed for inhibition of wild-type BRAF . Constitutive activation of BRAF V600E may also obviate the need for binding of cofactors normally required for MAPK activation, again leading to enhanced accessibility of

inhibitors to mutant BRAF. In addition, in melanoma cells with wild-type BRAF, treatment with selective RAF inhibitors leads to a paradoxical increase in MAPK signaling and activation of ERK. Similarly, in mouse models of melanoma driven by RTKs or bearing mutations of RAS (upstream of RAF in the MAPK pathway) treatment with selective RAF inhibitors stimulated tumor growth and led to development of secondary malignancies . This is because inhibition of BRAF in these wild-type BRAF cells allows increased signaling through CRAF, thereby allowing continued activation of the pathway. Of note, the development of cutaneous squamous cell carcinomas, most frequently of keratoacanthoma subtype, as a side effect of treatment with vemurafenib is a result of MAPK signaling through CRAF in cells with a pre-exiting upstream *RAS* mutation .

Not all patients with $BRAF^{V600E}$ melanoma respond to selective inhibition with vemurafenib or dabrafenib . Even in patients with $BRAF^{V600E}$, development of resistance to single agent vemurafenib or dabrafenib occurs in most patients within months. Several mechanisms of acquired resistance to vemurafenib and other selective RAF inhibitors have been identified to date (Figure 2). Some result in reactivation of the MAPK pathway, but to date, no secondary mutations (i.e. gatekeeper mutations) in the $BRAF^{V600}$ kinase have been identified . Instead, mechanisms of MAPK reactivation result from gene amplification of the mutant $BRAF^{V600}$ , splice variants of BRAF resulting in a smaller protein with increased ability to signal , secondary mutations in *NRAS* such as Q61K and development of mutations or deletions in *MEK1* . In addition, some resistant cell lines upregulate cancer osaka thyroid (COT or MAP3K8) signaling . Other mechanisms of resistance lead to enhanced cell signaling through pathways other than the MAPK. Examples include upregulation of RTKs such as the platelet-derived growth factor beta (PDGFRβ), or the insulin growth factor receptor 1 (IGF1R), or deletions of PTEN or mutations in PIK3CA or AKT . These all lead to enhanced PI3K/AKT/mTOR signaling rather than reactivation of the MAPK pathway. These mechanisms of acquired resistance appear to

develop mostly in a mutually exclusive manner. These resistance mechanisms have been corroborated clinically, in which these mutations or phenotypes have been identified in samples derived from patients treated with BRAF inhibitors.

**Beyond inhibition of mutated BRAF**

Knowledge of the mechanisms underlying resistance to vemurafenib, its analogs, or dabrafenib provide an important basis for developing rational strategies to treat patients who do not have *BRAF*$^{V600}$ mutated melanoma, patients with *BRAF*$^{V600}$ mutated melanoma who do not respond to BRAF inhibitors, or to treat patients who progress on these therapies. Data evaluating the susceptibility of cell lines derived from melanomas with acquired resistance to vemurafenib demonstrated that susceptibility to a MEK inhibitor was dependent on the mechanism of resistance . While initial *BRAF*$^{V600}$ melanoma cell lines were sensitive to both vemurafenib and the MEK inhibitor, many developed cross-resistance to both inhibitors. However, cell lines with the acquired *NRAS*$^{Q61}$ mutations remained sensitive to MEK inhibitor, demonstrating the continued dependence on the MAPK pathway for driver oncogenic signaling. The cell lines with RTK upregulation as the mechanism of acquired resistance did not respond to the addition of the MEK inhibitor because they use an alternative survival pathway through PI3K/AKT/mTOR. These RTK-mediated acquired resistance cell lines were indeed sensitive to the addition of an AKT inhibitor or rapamycin in combination with vemurafenib. Thus, these data demonstrate that acquired resistance to BRAF inhibitors may be overcome or partially overcome *in vitro* by addition of either a MEK inhibitor or an inhibitor of the AKT/mTOR pathway, depending on the mechanism of resistance. Similarly, cell lines resistant to dabrafenib remain sensitive to an inhibitor of PI3K/AKT/mTOR .

**Combining Inhibition of RAF with other MAPK inhibitors**

Given that MAPK dependence often persists even after resistance to BRAF inhibitors develops, several clinical trials evaluated MEK inhibitors, alone or in combination with type I RAF inhibitors.

In a Phase I dose escalation study of the MEK inhibitor, trametinib, Infante et al. reported a maximum tolerated dose of 2 mg daily. Dose limiting toxicities included rash, diarrhea and central serous retinopathy; 80% (165/206) of patients developed rash or acneiform dermitis and and diarrhea was seen in 42% of patients . Trametinib was evaluated in a multi-center, international Phase III study in V600E or V600K mutant melanoma patients who were BRAF or MEK inhibitor naïve. Patients were randomized 2:1 to trametinib or chemotherapy (dacarbazine or paclitaxel) and a statistically significant improvement in investigator-assessed PFS [HR 0.47 (95% CI: 0.34, 0.65); $p < 0.0001$] for trametinib was seen compared to chemotherapy. Objective response rate was 22% (95% CI, 17-28) for trametinib compared to 8% (95% CI, 4-15). Median PFS was 4.8 months for trametinib and 1.5 months for chemotherapy and the 6-month overall survival rate was 81% for trametinib, versus 67% for chemotherapy . Given these findings, the FDA approved trametinib for $BRAF^{V600E/K}$ mutant melanoma in May 2013.

In patients who had previously progressed on BRAF inhibitors, no responses to trametinib monotherapy were observed among 40 enrolled patients . However, addition of the MEK inhibitor cobimetinib to vemurafenib in patients who progressed on BRAF inhibitors resulted in modest responses: 10 of 66 patients (15%) who progressed on a BRAF inhibitor responded when cobimetinib was added. Median PFS was 2.8 months. In contrast to the modest responses in BRAF-inhibitor pretreated patients, among the 63 BRAF inhibitor-naïve patients evaluated, confirmed objective responses were seen in 87% (55/63), with 10% complete responders. The median overall survival was 13.7 months . Furthermore, the combination of

vemurafenib and cobemetinib resulted in a statistically significant increase in PFS compared to vemurafenib plus placebo (9.9 vs 6.2 months, HR 0.51; 95% CI 0.39-0.68, p<0.001) .

Similar results have been observed for the combination of dabrafenib and trametinib. A phase I/II clinical trial of dabrafenib with trametinib as first-line therapy for mutant BRAF tumors demonstrated that the combination was tolerated at the full doses used in monotherapy, though rates of pyrexia were significantly higher (71% for the combination vs. 26% for dabrafenib alone) . Furthermore, in the seminal Phase III study by Robert, et al. the median PFS for dabrafenib and trametinib was 11.3 months, compared to 7.3 months for vemurafenib. 12-month OS rate was also improved: 72% (95% CI, 67-77) for the combination vs. 65% (95% CI 59-70). Interestingly, because MEK inhibition blocks paradoxical MAPK inhibition by BRAF inhibitors , the incidence of squamous cell carcinomas with the combination therapy was markedly reduced. Cutaneous SCC were reported in 7% of patients treated with dabrafenib and trametinib, compared to 19% for dabrafenib and . Robert, et al. reported a 1% for the combination compared with 18% for vemurafenib monotherapy , indicating that dual inhibition of the MAPK pathway circumvents a common adverse event seen with monotherapy with BRAF inhibitors . With the statistically significant improvement in durable objective responses afforded by combination MAPK therapy, in 2014, the FDA granted accelerated approval to dabrafenib and trametinib for combination therapy for *BRAF*$^{V600E/K}$ metastatic melanoma.

Inhibition of MEK or ERK may also be an effective strategy for tumors that are MAPK driven in a BRAF-independent fashion. Preclinical work in melanoma cell lines with mutant NRAS demonstrated that while these were relatively insensitive to vemurafenib, treatment with a MEK inhibitor potently inhibited growth of tumor cells and resulted in decreased phosphoERK . Falchook et al. reported a 10% response rate for trametinib in BRAF-wild-type melanoma. Furthermore, a Phase II study of the MEK 1/2 inhibitor binimetinib reported six of 30 (20%) with

NRAS-mutated melanoma had a partial response to treatment. Among BRAF-mutant melanoma, eight of 41 (20%) of patients responded . The possibility that binimetinib may be effective in NRAS-mutant melanoma is being further explored in a randomized Phase III clinical trial with dacarbazine as the control arm (NCT01763164). Other MEK inhibitors that have been or are currently under clinical investigation as single agents or combinations include TAK733 (NCT00948467), and selumetinib (NCT01974752). ERK inhibitors demonstrated promising preclinical activity in both BRAF-mutant and wild-type melanoma and early phase clinical trials in BRAF-, NRAS- and MEK-mutated cancers are ongoing (NCT01781429).

**Combining inhibition of BRAF with PI3K/AKT/mTOR inhibitors**

As described above, PI3K/AKT/mTOR pathway activation is another important pathway driving pathogenesis in BRAF wild-type melanomas as well as in acquired resistance in BRAF mutant melanoma. Preclinically, several published studies demonstrate the utility of inhibition of PI3K/AKT/mTOR in melanomas with acquired resistance to vemurafenib or dabrafenib . Thus, several clinical trials of combining PI3K/AKT/mTOR inhibitors with MAPK pathway inhibitors such as BRAF or MEK inhibitors are currently ongoing or planned (NCT 1941927, NCT01902173, NCT01021748, NCT01519427, NCT01363232). One key question is whether inhibiting two key cell signaling pathways simultaneously will be tolerable at doses necessary for anti-tumor effect.

**Targeted therapy in combination with immunotherapy**

Melanoma has long been considered an immunosensitive tumor. Data include the finding that melanomas may undergo spontaneous regression and prolonged, durable responses may be seen after treatment of metastatic melanoma with high dose IL-2, interferon, anti-CTLA4 or anti-

PD1/l1. The impressive initial responses seen with BRAF inhibitors and the prolonged duration of responses that can be achieved with immunotherapy gives rise to an intriguing hypothesis that combining BRAF inhibitors with immunotherapy could augment the sensitization of immune cells to the cancer cells and result in long-lasting disease control . Preclinical data demonstrate that this approach may be feasible and effective. In two distinct mouse models of adoptive cell therapy for melanoma, preliminary results demonstrate that addition of vemurafenib yielded statistically significant improvements in tumor regression. Furthermore, at clinically relevant concentrations, vemurafenib neither affects the viability of lymphocytes from human peripheral blood mononuclear cells (PBMCs) nor does it significantly impair lymphocyte antigen recognition or cytotoxic activity , indicating that addition of vemurafenib should not adversely affect the efficacy of immunotherapy. Several clinical trials of PDL1/PD1 antibodies combined with BRAF and or MEK inhibitors are currently accruing (NCT02130466 and NCT02027961) .

**Conclusions**

The recognition of the key role of the MAPK pathway, and of $BRAF^{V600}$ in particular, in driving oncogenesis in melanoma was a pivotal breakthrough allowing identification of the type I RAF inhibitors like vemurafenib or dabrafenib as effective therapy. While initially effective, however, primary resistance or development of acquired resistance to these targeted therapies remains a significant issue. Several mechanisms of resistance have been identified and clinical trials are ongoing to evaluate the efficacy of other MAPK inhibitors or inhibitors targeting other key signaling pathways that are altered in melanoma. Combining targeted therapies or using targeted therapies in conjunction with immunotherapy may provide additional treatment options for this disease.

**Author Disclosures**

Deborah Wong has no conflicts of interests to declare.

Antoni Ribas has served as consultant for Amgen, Genentech-Roche, GSK, Merck and Novartis with the honoraria paid to UCLA.

**Figure Legends**

**Figure 1. Overview of the MAPK pathway.** Activation of a cell surface RTK such as cKIT leads to sequential phosphorylation and activation of proteins in the MAPK pathway: RAS, RAF, MEK and ERK. ERK activation then mediates phosphorylation of key proteins involved in cellular proliferation and other events.

**Figure 2. Mechanisms of Resistance to RAF Inhibitors.** Resistance to BRAF inhibitors by $BRAF^{V600}$ melanoma may occur via mechanisms that reactivate the MAPK pathway (mutations in NRAS or MEK, upregulation of COT pathway, upregulation of $BRAF^{V600}$ gene expression or generation of BRAF splice variants) or upregulation of RTKs that signal via pathways such as AKT/PI3K/mTOR.

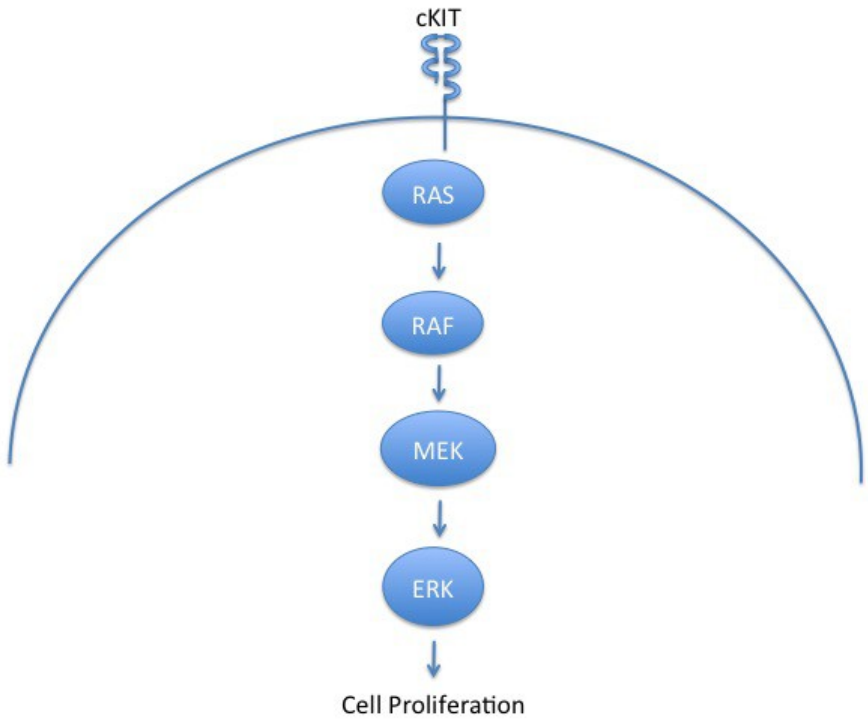


**Figure 1.**

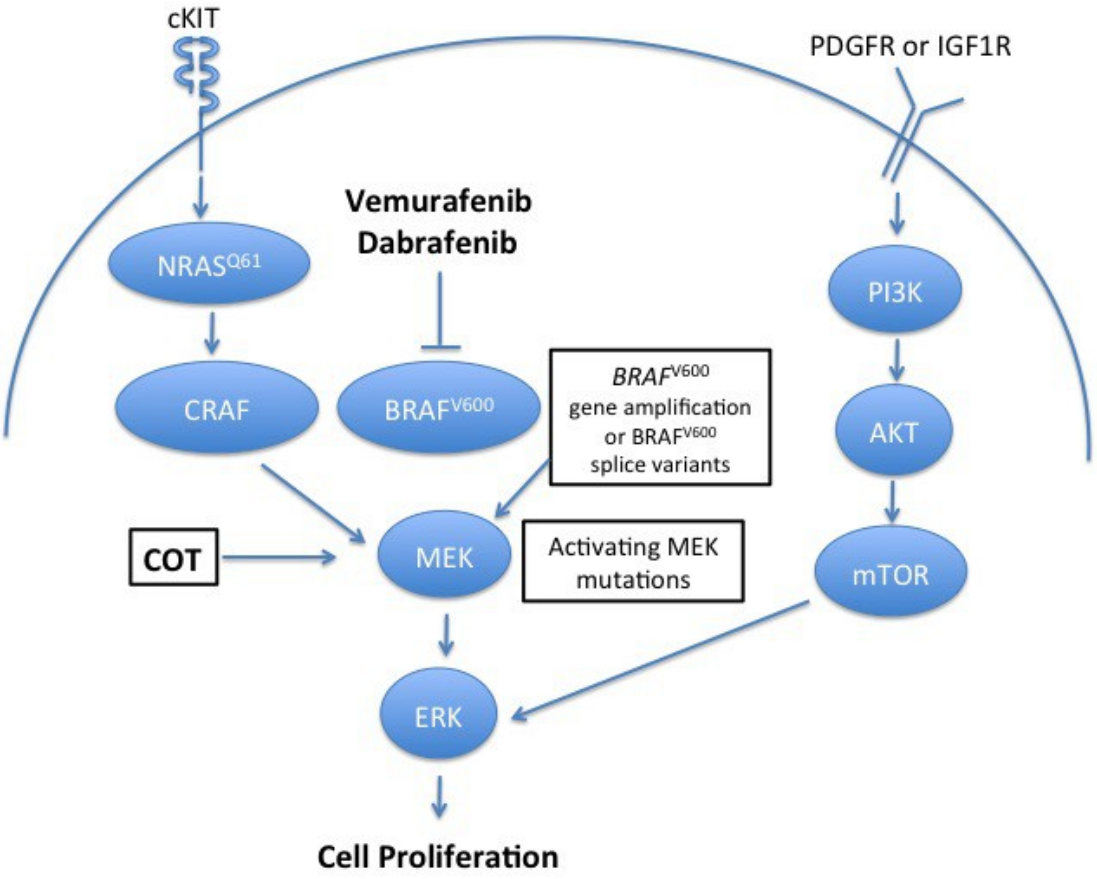
cKIT
PDGFR or IGF1R
Vemurafenib
Dabrafenib
NRAS$^{Q61}$
PI3K
CRAF
BRAF$^{V600}$
*BRAF*$^{V600}$ gene amplification or BRAF$^{V600}$ splice variants
AKT
COT
MEK
Activating MEK mutations
mTOR
ERK
Cell Proliferation

**Figure 2.**

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