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Resistance to RAF inhibitors revisited

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Abstract

In early 2011, we reviewed the initial success of the RAF inhibitor, vemurafenib, in mutant V600 BRAF melanoma patients. It was soon evident that the response to RAF inhibitor is heterogeneous and that the short-term benefits are burdened by the development of resistance. The field has progressed rapidly with the FDA-approval of vemurafenib and the development of other RAF and MEK inhibitors. Despite these advances, the issue of RAF inhibitor resistance remains. Here, we review recent clinical advances in the field, the growing number of resistance mechanisms, preclinical evidence for combinatorial trials using RAF inhibitors as the building blocks, and the new challenges that are arising.

Keywords

BRAF; dabrafenib; ERK1/2; melanoma; vemurafenib

Introduction

The introduction of RAF inhibitors has dramatically changed the treatment options for the ~50% of melanoma patients that harbor V600 BRAF mutations. However, as with other targeted therapies, mechanisms of primary/intrinsic and acquired resistance exist. We will initially review the phase 2 and phase 3 trial results with vemurafenib and the findings with the selective RAF inhibitor, dabrafenib, and new MEK inhibitors. Second, we will outline resistance mechanisms and elaborate on our model of compensatory pathways being important in the primary response and re-activation of the MEK-ERK1/2 pathway (Figure 1).

Clinical trials lead the way

The findings from the phase 1 vemurafenib trials in melanoma were a breakthrough for the field (Flaherty *et al*., 2010) and were rapidly supported by the phase 2 and 3 findings. In the

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phase 2 trial of vemurafenib, 132 melanoma patients were enrolled who were confirmed for BRAF V600 status but had not previously received other drug regimens (Sosman *et al*., 2012). Patients were dosed with 960 mg of vemurafenib taken orally twice daily. The study yielded promising results with a confirmed overall response rate of 53% and median progression free survival (PFS) of 6.8 months. Notably, 25% of patients tested demonstrated a PFS of greater than 12.9 months. The phase 3 trial comprised of 675 therapy-naïve patients and directly compared the efficacy of vemurafenib to the previous first line therapy, dacarbazine (Chapman *et al*., 2011; Chapman *et al*., 2012b). Patients testing positive for a stage IIIC or IV BRAF V600 melanoma were randomly assigned to the vemurafenib group or the dacarbazine group. A dramatic difference between overall response rates was observed: 48% for vemurafenib versus 5% for dacarbazine. Median overall survival rates were calculated to be 13.2 months, and 9.6 months for vemurafenib and dacarbizine, respectively (Chapman *et al*., 2012b). Additionally, vemurafenib provided a median PFS of 5.3 months compared to 1.6 months with dacarbazine.

Other selective RAF inhibitors including dabrafenib (GSK2118436) with preference for the V600 mutant form of BRAF have also been developed. Initial clinical data with dabrafenib was promising (Hauschild *et al*., 2012) and this RAF inhibitor was given FDA approval in May 2013 for V600E BRAF-driven melanoma. An open-labeled randomized phase 3 trial was conducted with 250 patients randomly selected to receive either dabrafenib (150 mg twice daily) or dacarbazine. A recent update of this trail showed that dabrafenib elicited a median PFS of 6.9 months compared to 2.7 months with dacarbazine (Hauschild *et al*., 2013). It was also found that dabrafinib provided an overall survival rate of 18.2 months compared to 15.6 months with dacarbazine treatment. Importantly in a phase 2 trial of dabrafenib, patients with previously untreated or treated brain metastases exhibited response rates of 22% and 31%, respectively (Long *et al*., 2012). These findings are notable given the frequency of brain metastasis in melanoma.

Next generation MEK inhibitors with improved pharmacokinetic properties have also shown promise as a treatment option for mutant BRAF melanomas leading to the FDA approval of trametinib (GSK1120212) in May 2013 for the treatment of V600E/K BRAF melanoma. In a phase I trial, trametinib provided 5.7 months of PFS in V600 mutant BRAF metastatic melanoma patients compared to 2.0 months in wild type BRAF patients (Falchook *et al*., 2012). In a phase 3 study of V600E/K BRAF patients, median PFS was 4.8 months in the trametinib-treated cohort compared to 1.5 months with standard chemotherapy (Flaherty *et al*., 2012b). Trametinib has also been tested in a larger scale phase II study comprising of 2 arms, a cohort of patients having mutant BRAF melanoma progressing under BRAF inhibitor treatment and a cohort with mutant BRAF having had no previous BRAF targeted treatments. Results demonstrate that trametinib is quite effective against BRAF inhibitor naïve patients exhibiting a PFS of 4.0 months. However, PFS was only 1.8 months in the cohort of patients who have been treated previously with a selective BRAF inhibitor (Kim *et al*., 2013). In the latter, BRAF inhibitor treatment was stopped prior to trametinib treatment perhaps suggesting that MEK inhibitors alone are not able to sufficiently inhibit the ERK1/2 pathway in resistant patients.

Although it might seem counterintuitive to target the same pathway at multiple points, evidence from the vemurafenib alone trials indicated a need for >80% inhibition of phospho-ERK1/2 for clinical effect (Bollag *et al*., 2010). Thus, combinational therapies of RAF and MEK inhibitors are currently being evaluated. A phase I/II trial of dabrafenib and trametinib was conducted to address the safety and the possibility of an adverse drug interaction (Flaherty *et al*., 2012a). Median PFS was improved by 3.6 months in patients receiving dabrafenib concurrently with a 2 mg/day dose of trametinib when compared to patients only being treated with dabrafenib. Additionally, a partial or complete response was observed in 76% of the dual-therapy patients, while a response rate of 54% was elicited in patients receiving the dabrafenib monotherapy. The combined treatment regimen is well tolerated by patients and even reduces the risk of cutaneous squamous cell carcinoma/keratoacanthoma (cuSCC/KA) from 19% in a dabrafenib monotherapy to 2–7% (depending on the concentration of MEK inhibitor used). In the studies with trametinib alone, there was no induction of keratoacanthoma (Kim *et al*., 2013).

Immunological effects of RAF inhibitors

While BRAF inhibitors block MEK-ERK1/2 activation in cells possessing V600E BRAF, a paradox exists in that RAF inhibitors cause induction of the pathway in wild-type BRAF cells with high RAS activity (Kaplan *et al*., 2010). This paradoxical ERK1/2 activation can induce cuSCC/KA, adenomas and in one case leukemia (Callahan *et al*., 2012; Chapman *et al*., 2012a; Su *et al*., 2012b). However, the same paradoxical signaling has some positive effects. One example is vemurafenib stimulation of the immune response. Koya *et al*. describe that tumor-infiltrating lymphocytes possessed higher secretion of the potent immunostimulant, interferon-γ, following vemurafenib treatment and this observation correlated with an increased intrinsic cytotoxic ability (Koya *et al*., 2012). Furthermore, they demonstrated an enhanced anti-tumor effect against a subcutaneously implanted, mouse-derived V600E BRAF melanoma line using vemurafenib in combination with adoptively transferred splenocytes designed to target the melanocyte marker gp100. Vemurafenib paradoxically enhanced ERK1/2 signaling in these cells, and the authors believe this may be the mechanism responsible for the enhanced immune response and contribute to the overall anti-tumoral effect.

The effect of ERK1/2 pathway inhibitors on the immune system was also investigated by Wargo and colleagues. This group demonstrated that both RAF and MEK inhibitors increased the expression of melanocyte differentiation antigens (MDAs) on melanoma cells and that these MDAs are targets for T-lymphocytes (Boni *et al*., 2010). The group further demonstrated that the pantropic effects of the MEK inhibitors depress the efficacy of the T-lymphocytes themselves, but importantly, that selective RAF inhibitors are not suppressive. These data raise important differences between RAF inhibitor alone versus MEK inhibitor-containing treatments and suggest that selective RAF inhibitors combined with immunotherapy may be an efficacious approach to melanoma therapy.

A phase I trial was conducted based on some of these data utilizing a dual treatment of vemurafenib combined with the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) blocking antibody, ipilimumab. However, this study was ended with patients experiencing

significant hepatotoxic effects, and trial organizers needed to curtail drug exposure (Ribas *et al*., 2013). While these results were disappointing, there have been significant other advances in the immunotherapy field utilizing antibodies against programmed death 1 (PD-1) (Hamid *et al*., 2013; Wolchok *et al*., 2013). Therefore, trials testing the combination of RAF inhibitor and anti-PD-1 antibodies are likely.

Mechanisms of resistance to RAF inhibitors

As noted above, the durable benefit provided by RAF inhibitors is limited by resistance. The mechanisms of resistance fall into two broad categories. Intrinsic/primary resistance is displayed by approximately 50% of patients- ~15% of patients show no tumor shrinkage in response to vemurafenib, while ~35% of patients achieve a degree of tumor shrinkage that is not sufficient to meet the RECIST criteria for a partial response (Chapman *et al*., 2011; Flaherty *et al*., 2010; Sosman *et al*., 2012). The other 50% of the patients initially respond (>30% tumor shrinkage) to RAF inhibitor but subsequently develop progressive disease associated with acquired/secondary resistance to RAF inhibitor. These two categories are not mutually exclusive since nearly all responders have remaining disease and, thus, may display intrinsic resistance. Here, we review the mechanisms associated with each category.

Reactivation of ERK1/2 pathway associated with acquired resistance

Multiple studies have treated mutant BRAF melanoma lines that are susceptible to BRAF perturbation with vemurafenib for prolonged periods *in vitro* until drug resistant colonies develop. From these studies, it is clear that numerous mechanisms of resistance can develop, even from within a single cell line (Gowrishankar *et al*., 2012). A consistent theme among these mechanisms is ERK1/2 pathway re-activation in the presence of RAF inhibitor. Several of these mechanisms have been validated in patient samples.

In 2010, the initial studies on RAF inhibitor resistance from Nazarian and colleagues elucidated two mutually exclusive mechanisms to negate the blockade of BRAF V600E signaling. The first was up-regulation and activation of the receptor tyrosine kinase (RTK), PDGFR-β, and the second was expression of mutant Q61 NRAS (Nazarian *et al*., 2010). More recently, NRAS mutations were discovered in 4 of 19 patient samples (Poulikakos *et al*., 2011), validating NRAS mutations coexisting with BRAF V600E as a frequent mechanism of resistance. While NRAS is the most frequent form of RAS that is mutated in melanoma, HRAS and KRAS mutations occur in 1% and 2% of patients, respectively. These may also be important in the setting of acquired resistance to RAF inhibitors, since acquired KRAS mutations have been reported in vemurafenib-treated A375 V600E melanoma cell line (Su *et al*., 2012a).

A second mechanism of resistance that relies on ERK1/2 re-activation is alternative splicing of V600E BRAF. Poulikakos *et al*. detected a 61-kDa form of V600E BRAF, which lacked exons 4–8 (encoding the RAS binding domain of BRAF) from *in vitro* resistant cell lines (Poulikakos *et al*., 2011). This V600E BRAF variant possesses a markedly higher dimerization property irrespective of RAS status and strongly activates MEK and ERK1/2 in the presence of vemurafenib. Additionally, V600E BRAF copy number amplification has also been implicated as a mechanism of resistance. Whole exome sequencing of twenty

patients comparing base-line melanoma to that of a RAF inhibitor progressing tumor revealed that 20% (4 out of 20) harbored increased V600E BRAF copy number, which ranged from 2.2- to 12.8-fold. Further profiling of the patients with increased copy number did not identify other known mechanisms of vemurafenib resistance, such as acquired NRAS mutation or RTK amplification, raising the possibility that V600E BRAF amplification alone is sufficient for BRAF inhibitor resistance (Shi *et al*., 2012b). In a small percentage of cases, activating MEK1 mutations have also been implicated as a mechanism of vemurafenib resistance. Targeted sequencing of a cancer gene panel revealed an activating mutation in the RAF target, MEK1 (Wagle *et al*., 2011). A tumor biopsied from a patient whom became resistant to vemurafenib treatment harbored a cysteine to serine mutation at codon 121 (C121S) of MEK1. The phosphorylation of ERK1/2 in cells expressing C121S MEK1 was elevated and this variant was permissive for growth in vemurafenib-treated mutant BRAF cell lines. However, the significance of other MEK1 mutations has been called into question since P124S and I111S alterations have been detected in pre-treatment samples and do not provide resistance to RAF inhibitors (Shi *et al*., 2012a). The frequency of acquired MEK mutations is likely to become an area of active interest as RAF/MEK inhibitor combinations are more frequently utilized.

Up-regulated RTK signaling associated with acquired resistance

Up-regulation of RTKs has been frequently linked to resistance to targeted inhibitors. As previously reviewed (Aplin *et al*., 2011), up-regulation of PDGFRβ and IGF-1R expression is found in vemurafenib-resistant melanoma cell lines evolved *in vitro* and in patient tumor samples following disease progression. Further studies are starting to shed light on the mechanisms of resistance provided by RTKs. In follow-up work on PDGFRβ, Lo and colleagues showed that the inhibition of ERK1/2 phosphorylation by vemurafenib in PDGFRβ-resistant cells is transient with a robust rebound of phospho-ERK1/2 within 24 hours (Shi *et al*., 2011). The contribution of this ERK1/2 signal rebound to resistance and whether PDGFRβ is involved in regulating this rebound signal remain to be determined. In addition to the ERK1/2 pathway, PDGFRβ may also signal via phosphatidylinositide 3-kinase (PI3K)/AKT. In support of this, Shi *et al*. reported increased phosphorylation of AKT and its downstream effector, p70S6K, in PDGFRβ-mediated resistant melanoma cells (Shi *et al*., 2011). Based on these findings, the authors have proposed to use a combination of MEK inhibitor, AKT inhibitor, and mTORC inhibitor to overcome PDGFRβ-mediated resistance. Although this mechanism of resistance depends on PDGFRβ for growth, PDGFRβ-overexpressing cells are surprisingly resistant to the PDGFRβ inhibitor, imatinib. This is not due to a failure of the drug to inhibit PDGFRβ kinase activity as the phosphorylation of PDGFRβ was successfully blocked by imatinib (Shi *et al*., 2011). These findings raise the possibility that PDGFRβ functions as a scaffold protein in addition to its kinase activity to promote resistance.

Other studies have shed light on how vemurafenib-resistant cells that express high PDGFRβ levels evade apoptosis downstream of re-activation of ERK1/2 signaling. Vemurafenib induces apoptosis through up-regulation of two pro-apoptotic BH3-only proteins, Bim-EL and Bmf (Shao and Aplin, 2010). Studies in a subset of vemurafenib-resistant cells which display high levels of PDGFRβ show that the up-regulation of Bim-EL and Bmf is silenced

(Shao and Aplin, 2012). Importantly, the repression of these genes is largely ERK1/2-independent. Although the mechanism for Bmf repression is not clear, Bim-EL is silenced epigenetically. Treatment with the histone deacetylase (HDAC) inhibitor, vorinostat (suberoylanilide hydroxamic acid), reversed the repression of Bim-EL and re-sensitized resistant cells to vemurafenib (Shao and Aplin, 2012), suggesting that combinational treatment of vemurafenib with HDAC inhibitors may prove useful in fighting resistance. *In vitro* work done by Peter Hersey's group has demonstrated a strong synergism in the induction of apoptosis when vemurafenib and HDAC inhibitors are administered to V600E mutant BRAF melanoma cells (Lai *et al*., 2012). Given the importance of Bim-EL in apoptosis, it may serve as a good maker for vemurafenib responsiveness and resistance. Indeed, Faber *et al*., have shown that Bim expression predicts responsiveness to kinase inhibitor in treatment-naive cancers (Faber *et al*., 2011).

Mechanisms of primary/intrinsic resistance

Approximately 15% of patients treated with vemurafenib exhibit disease progression. In cell-based assays, loss or inactivation of key tumor suppressors contribute to this intrinsic resistance to vemurafenib. Both the retinoblastoma susceptibility gene (pRB) and the lipid phosphatase, phosphatase and tensin homolog (PTEN), have been implicated in intrinsic mutant BRAF inhibitor resistance. V600E BRAF melanoma cell lines null for PTEN tend to be more resistant to vemurafenib treatment compared wild type PTEN counterparts (Paraiso *et al*., 2011; Xing *et al*., 2012). Similar findings have been observed in response to MEK inhibitors (Gopal *et al*., 2010) and are consistent with findings that activated AKT3 is sufficient to provide resistance to PLX4720-mediated apoptosis (Shao and Aplin, 2010). This apparent decrease for BRAF oncogene addiction in mutant PTEN tumors was also demonstrated in the clinic. Nathanson and colleagues have evidence that in V600E BRAF melanoma patients with wild type PTEN, dabrafenib may elicit a longer progression free survival when compared to patients harboring at least one mutated allele of PTEN (32.1 weeks compared to 18.3 weeks, respectively) (Nathanson *et al*., 2013). While this set of data did not reach a statistically significant difference ($p= 0.066$), a larger sample size may confirm their findings.

Alterations in RTK signaling have been implicated in the primary resistance setting as well as in acquired resistance. Wilson and colleagues demonstrated that addition of hepatocyte growth factor (HGF) to V600E BRAF melanoma cell lines was sufficient to confer resistance to vemurafenib through c-MET receptor activation of ERK1/2 (Wilson *et al*., 2012). This resistance mechanism was attenuated by the c-MET inhibitor, crizotinib, *in vitro* and in a xenograft model. Furthermore, in patients, high serum HGF levels prior to a vemurafenib treatment is predictive of a shorter PFS and reduced overall survival (Wilson *et al*., 2012). A related study examined RAF inhibitor resistance as a result of co-culturing mutant BRAF melanoma tumor cells with stromal cells. It was found that stromal cells producing high levels of HGF conferred the strongest resistance to vemurafenib through activation of c-MET and downstream ERK1/2 signaling (Straussman *et al*., 2012).

In addition to pre-existing primary resistance mechanisms, tumor cells may elicit an adaptive response as an escape mechanism. Recently, our group has demonstrated adaptive

up-regulation of the stemness factor, FOXD3, in response to vemurafenib treatment (Abel and Aplin, 2010). This drug-induced increase in FOXD3 modifies the apoptotic response to vemurafenib such that FOXD3 knockdown in intrinsically resistant cells dramatically enhanced vemurafenib-induced apoptosis (Basile *et al*., 2012). Conversely FOXD3 expression in RAF inhibitor-sensitive cells provided protection (Basile *et al*., 2012). One target of FOXD3 is the RTK, v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (ERBB3)/human epidermal growth factor receptor (HER) 3. Activation of ERBB3 and its signaling to AKT were enhanced in vemurafenib-inhibited melanoma cells *in vitro*, melanoma cell xenografts in mice, and in patients samples from RAF inhibitor clinical trials (Abel *et al*., 2013). ERBB3 signaling was dependent on its related family member, ERBB2, and targeting ERBB3-ERBB2 signaling either molecularly or with lapatinib in combination with the RAF inhibitor, PLX4720, reduced tumor burden and extended latency of tumor regrowth *in vivo* versus PLX4720 alone. These results suggest that enhanced ERBB3 signaling may serve as a mechanism of adaptive resistance to RAF and MEK inhibitors in melanoma and that co-targeting this pathway may enhance the clinical efficacy and extend therapeutic duration of RAF inhibitors.

A separate study focused on RAF inhibitors causing a relief of feedback inhibition of RTK signaling and re-setting of the ERK1/2 pathway in a subset of mutant BRAF melanoma cells (Lito *et al*., 2012). Lito and colleagues demonstrated an ERK1/2 activation rebound in the presence of RAF inhibitors associated with activated RTK, specifically ERBB/HER family members. The level of reactivation is modest and/or cell line-dependent since other studies have not detected reactivation in mutant BRAF melanoma cells (Corcoran *et al*., 2012; Montero-Conde *et al*., 2013). They correlate this RTK-driven ERK rebound with an elevated Ras-GTP bound state, which induces the formation of RAF inhibitor-resistant RAF dimers. These RAF dimers can then stimulate MEK and ERK1/2, irrespective of RAF inhibitor. The group also showed that blockade of the elevated RTK signaling allowed mutant BRAF melanoma cells to become sensitized to RAF inhibitors; however, pathways in addition to ERK1/2 are targeted in this scenario. Nevertheless, these findings add to the growing evidence for up-regulated RTK signaling as an adaptive response to RAF inhibitors. Future avenues will have to investigate what determines the range of adaptive response and optimize co-targeting approaches.

Adaptive responses may also impact on additional pathways. Haq and colleagues described an adaptive response to ERK1/2 pathway suppression that leads to the upregulation of mitochondrial synthesis and oxidative metabolism (Haq *et al*., 2013). They found that when RAF inhibitors are used to suppress mutated BRAF, the transcription factor, microphthalmia-associated transcription factor (MITF), is upregulated, and controls expression of the mitochondrial regulator peroxisome proliferator-activated receptor γ, coactivator 1α (PGC1α). PCG1α was sufficient for the observed increase in mitochondrial number and output, and was associated with enhanced oxygen consumption. The group also demonstrated that mutant BRAF cells treated with RAF inhibitors are susceptible to mitochondrial uncouplers suggesting that ERK1/2 suppressed melanoma cells are addicted to oxidative phosphorylation. Indeed, this idea was substantiated in xenograft models and

ushers in a new possibility of treatment options by combining ERK1/2 pathway inhibitors with mitochondrial uncouplers.

The notion of oxidative phosphorylation state as a marker for intrinsic resistance was also recently explored by the Herlyn group. They demonstrated that a small subset of melanoma cells contain a high level of the H3K4 demethylase, jumonji AT rich interactive domain 1B (JARID1B), and these slow-cycling cells are inherently more resistant to chemotherapies, as well as targeted therapies such as vemurafenib (Roesch *et al*., 2013). They correlated high JARID1B expression with elevated levels of proteins associated with mitochondrial respiration. Similar to Haq *et al*., the Herlyn group also found that inhibition of mitochondrial function further sensitized cells to therapeutics. In fact, they demonstrated that various mitochondrial blockers ablated the formation of the slow-cycling JARID1B^{high} sub population, suggesting that intrinsic drug resistance is associated with elevated oxidative phosphorylation.

Pre-clinical basis for additional combinatorial strategies

The introduction of vemurafenib and dabrafenib has been a significant breakthrough in the melanoma field; and these inhibitors now represent the building blocks for combined targeted therapeutic strategies. Already clinical trials combining RAF inhibitors with other targeted agents have been shown to significantly increase PFS over RAF inhibitor monotherapy. As the clinical field quickly progresses, so do pre-clinical studies that form the basis for future combinations. Clinically effective ERK1/2 inhibitors are being developed and cell lines with an acquired MEK1 gate-keeper mutation are resistant to MEK inhibitors but susceptible to ERK1/2 inhibitor treatment (Hatzivassiliou *et al*., 2012). These data support the notion that multiple attacks on a linear pathway will be clinically efficacious.

Building upon the idea that RTK amplification and hyperactivation is a major mechanism of resistance, a study by Metzner *et al*. demonstrated that FGF ligands and receptors are expressed in high levels in melanoma cells (Metzner *et al*., 2011). The group demonstrated a synergistic effect in reduced proliferation and enhanced apoptosis when an FGF inhibitor, either SU5402 or PD166866, was combined with RAF inhibitor. The authors postulated that a greater level of clinical efficacy may be obtained if both the presumed tumor specific oncogene is targeted as well as a “universally hyperactivated” upstream molecule.

Several studies have examined the synergism/additive nature of targeting the PI3K/AKT pathway in combination with either RAF or MEK inhibitors in preclinical models. While many of these studies show promise *in vitro* (Table 1), their utility in patients is often burdened by toxicity issues. Xing and colleagues were able to demonstrate a synergism associated with melanoma apoptosis when combining a MEK inhibitor with a PI3K inhibitor (Xing *et al*., 2012). Furthermore, a recent phase II study of the MEK inhibitor, selumetinib, found that a low patient response rate is associated with high basal levels of phosphoAKT (Catalanotti *et al*., 2013). This further supports the rationale that stronger anti-tumoral efficacy will be obtained when multiple pathways are targeted.

Alternative treatment approaches

An alternative approach is to selective targeting of signaling pathways is to broadly attack resistance nodes, which arise as a result of vemurafenib treatment. Based on the observation that several of the aforementioned resistance mechanisms are mediated by client proteins heat shock protein 90 (HSP90), the Smalley group utilized the selective HSP90 inhibitor, XL888 (Paraiso *et al*., 2012). Their data demonstrate that upon XL888 treatment, various molecules known to have a role in RAF inhibitor resistance such as PDGFRβ, IGF1R, and CRAF are quickly degraded as a result of loss of HSP90 chaperone function. Ultimately, this leads to an enhanced susceptibility to apoptosis compared to a combined treatment of MEK and PI3K inhibition.

More recently, the McMahon and Stuart groups demonstrated efficacy when utilizing a “drug holiday” regimen in a xeongraft model (Das Thakur *et al*., 2013). With an on-again, off-again BRAF inhibitor treatment regimen, they were able to demonstrate tumor shrinkage during the periods of drug removal after the initial tumor relapse, suggesting a drug addiction. Over time, in the non-treated state, cells would adapt and begin to grow, however a second treatment wave of BRAF inhibitor would shrink the tumor again. They demonstrated a cyclical pattern of tumor growth/shrinkage, which was linked to BRAF inhibitor addiction.

Conclusions

Vemurafenib is one of the first successful small molecule inhibitors for personalized, targeted, cancer treatment; however, it will likely serve as a building block for further improvements to treatment. New studies have highlighted the benefits of utilizing a combined treatment regimen and it is likely that a dual or even a cocktail of selective inhibitor agents will emerge as the standard of melanoma care in the near future. There is now strong evidence to support combining inhibitors in the same linear pathway or attacking multiple deregulated proteins that primarily act in distinct signaling pathways. It is hoped that these combinatorial approaches will ultimately lead to a better patient outcome.

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Abbreviations

cuSCC	cutaneous squamous cell carcinoma
ERBB3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3
HDAC	histone deacetylase
HSP90	heat shock protein 90
HER	human epidermal growth factor receptor

HGF	hepatocyte growth factor
JARID1B	jumonji AT rich interactive domain 1B
KA	keratoacanthoma
MDA	melanocyte differentiation antigen
MITF	microphthalmia-associated transcription factor
PFS	progression free survival
PGC1α	peroxisome proliferator-activated receptor γ coactivator 1α
PI3K	phosphatidylinositide 3-kinase
PTEN	phosphatase and tensin homolog
RTK	receptor tyrosine kinase
RB	retinoblastoma

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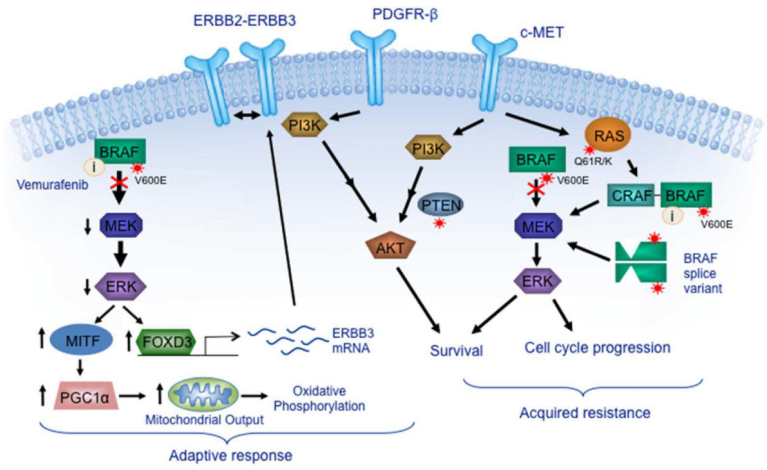

Figure 1.

Overview of resistance mechanisms to RAF inhibitors in mutant BRAF melanoma.

Table 1

RAF/MEK and PI3K/AKT combination studies

Combination	Significance	Citation
MEK inhibitor + PI3K/AKT inhibitor		
PD0325901 (MEKi) + PI-103 (PI3Kalpha inhibitor)	Synergism was found in combination treatment which strongly induced apoptosis in mutant BRAF/PTEN null melanoma cell lines	(Xing *et al.*, 2012)
Trametinib + GSK2126458 (PI3K/mTORi)	RAF inhibitor-resistant A375 and YUSIT1 cells were sensitive to MEK and PI3K inhibition	(Greger *et al.*, 2012)
AZD6244 (MEKi) + VIII (AKTi)	Multiple *in vitro*-derived and patient-derived resistant lines were synergistically affected by combination treatment.	(Atefi *et al.*, 2011)
GDC-0973 (MEKi) + GDC-0941 (PI3Ki)	Comparisons were made between xenograft growth rates of single agent compared to combination treatment. An additive effect on growth inhibition was observed when combining the inhibitors.	(Choo *et al.*, 2013)
RAF inhibitor + PI3K/AKT inhibitor		
Vemurafenib + MK-2206 (AKTi)	Dose escalation of MK-2206 with vemurafenib demonstrated a combinatorial inhibitory effect on *in vitro* proliferation. The combination treatment reduced cyclinD1 expression and upregulated p27Kip1 and Bim-EL.	(Su *et al.*, 2012a)
Vemurafenib + VIII (AKTi)	*In vitro*-derived and patient-derived resistant melanomas were found to be synergistically affected by combination treatment.	(Atefi *et al.*, 2011)