Targeting Mutant *BRAF* in Melanoma: Current Status and Future Development of Combination Therapy Strategies

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Abstract

The discovery of activating *BRAF* mutations in ~50% of all melanomas has proved to be a turning point in the therapeutic management of the disseminated disease. In this commentary we review the latest research delineating the role of mutant *BRAF* in melanoma initiation and progression and discuss the remarkable 10-year journey leading up to the recent FDA-approval of the small molecule BRAF inhibitor vemurafenib. We further outline the most recent findings on the mechanisms that underlie intrinsic and acquired BRAF inhibitor resistance and describe ongoing preclinical and clinical studies designed to delay or abrogate the onset of therapeutic escape. It is hoped that our evolving understanding of melanoma genetics and intracellular signaling coupled with a growing armamentarium of signal transduction inhibitors will lead to significant improvements in the level and durability of therapeutic response in metastatic melanoma.

Keywords

melanoma; BRAF; vemurafenib; GSK2118436; resistance

Introduction

Disseminated melanoma has a dismal prognosis and is almost completely resistant to therapeutic modalities such as chemotherapy and radiotherapy. The 2002 discovery of activating mutations in the serine/threonine kinase BRAF (v-raf murine sarcoma viral oncogene homolog B1) in approximately 50% of all melanomas kick-started a targeted therapy "arms race" which in under 10 years led to the FDA-approval of the BRAF inhibitor vemurafenib[1,2]. Together, these studies have set a new benchmark for the rapid development of genome-specific anti-cancer agents. In this commentary we will review the latest developments in our understanding of the role of BRAF signaling in melanoma pathogenesis and will discuss the preclinical and clinical development of small molecule inhibitors of the

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BRAF/MEK/ERK signaling pathway. We will further outline the challenges that remain in the effective clinical translation of BRAF/MEK/ERK inhibitors with a particular emphasis upon emerging strategies for the management of BRAF inhibitor resistance in the clinic.

The biology of mutated *BRAF* and the role of the MEK/ERK signaling pathway in melanoma development and progression

BRAF is a member of the Raf family of serine threonine kinases (ARAF, BRAF and CRAF), which are part of the Ras/Raf/MEK/ERK mitogen activated protein kinase (MAPK) signal transduction cascade. The MAPK pathway is a key mediator of growth signaling that links cell surface growth factor receptors (such a receptor tyrosine kinases; RTKs) to the increased transcription of genes required for cell cycle entry. Although mutations in *BRAF* have been described at a number of sites (see[3] for a comprehensive list), the majority, which account for >80%, result in the substitution of valine to glutamic acid (the *BRAF* V600E mutation)[1,4]. Acquisition of the V600E mutation destabilizes the inactive conformation of the BRAF kinase shifting the equilibrium to the active state[5]. Of the other *BRAF* mutations identified in melanoma, V600K, V600D/R are also common and represent 16% and 3% of all *BRAF* mutations, respectively[6]. In addition to melanoma, *BRAF* mutations are also common in many other cancers including papillary thyroid carcinoma, ovarian carcinoma and colorectal carcinoma[7].

Despite the well established role of *BRAF* mutations in cancer, equivalent activating mutations in either ARAF or CRAF are extremely rare[7]. The reasons behind this are still subject to debate but seem to be a consequence of the relative ease of BRAF activation (with only Ras-mediated membrane recruitment required) compared to the more complex process of *ARAF* and *CRAF* activation (which involves a number of priming phosphorylation events at multiple sites by Src and other as yet unidentified kinases[7]).

V600E-mutated *BRAF* is a *bona fide* melanoma oncogene, with its introduction leading to the malignant transformation of immortalized human melanocytes both *in vitro* and *in vivo*[8]. In transgenic mouse models the conditional, melanocyte-specific expression of *BRAF* V600E leads to spontaneous melanoma formation, but only in conjunction with the inactivation of the tumor suppressor phosphatase and tensin homolog (PTEN)[9]. This, together with data showing that introduction of mutant *BRAF* alone into primary human melanocytes leads to senescence, suggests that although mutated *BRAF* may be an initiating factor in melanomagenesis, other co-operating events are also required[10].

Although *BRAF* mutations are not ultraviolet (UV) radiation signature mutations, they have a tendency to occur on UV-exposed skin sites and are more prevalent in individuals with a poor tanning response[11]. There is also evidence that intermittent, rather than chronic sun-exposure is predictive for *BRAF* mutational status with *BRAF* mutant melanoma patients tending to be younger in age (<55 years old) with a lower cumulative UV exposure[12]. *BRAF* mutational status is also of prognostic value and is associated with inferior survival in the metastatic setting (8.5 months in BRAF wild-type vs 5.7 months for *BRAF* mutant melanoma)[13].

Much of the transforming activity of mutant *BRAF* is mediated through activation of the RAF/MEK/ERK pathway[1]. Signaling through the MAPK pathway drives the growth of melanoma cells through the upregulation of cyclin D1 expression and by downregulating the cell cycle inhibitor $p27^{KIP1}$. It also serves to increase melanoma cell survival by regulating the expression and function of a number of pro and anti-apoptotic proteins, such as BIM, BMF, BAD and Mcl-1[14–17] (Figure 1). Inhibition of BRAF or MEK signaling using either small molecule inhibitors or siRNA knockdown increases the expression of the pro-apoptotic BH3-only protein BIM which induces apoptosis by binding to and antagonizing

the activity of the pro-survival proteins Bcl-2, Bcl-w, Bcl-XL and Mcl-1[18,19]. BIM is known to exist as three spliceforms BIM-EL (extra long), BIM-L (long), and BIM-S (short), with BIM-S being the most cytotoxic isoform[19]. The BRAF/MEK/ERK pathway regulates BIM expression through phosphorylation at Ser69, leading to its proteasomal degradation and by differentially regulating BIM splicing[18,20]. Survival of melanoma cells is also controlled in part by the anti-apoptotic protein, Mcl-1, whose stability can also be regulated through the BRAF/MEK/ERK pathway[15] (Figure 1). A number of recent studies have further suggested a role for increased BMF (Bcl-2 modifying factor) expression in mediating the apoptotic response of melanoma cells treated with inhibitors of BRAF and MEK[14,21].

Constitutive activity in the BRAF/MEK/ERK pathway in melanoma drives the invasive and motile behavior of melanoma cells through the re-organization of the cytoskeleton, the activation of the cells' migratory machinery and the upregulation of matrix metalloproteinase expression[22,23]. Mechanistically, mutated *BRAF* downregulates the expression of the cyclic GMP phosphodiesterase PDE5A, which facilitates melanoma cell invasion by increasing intracellular levels of cGMP, leading to the release of cytosolic calcium and the phosphorylation of myosin light chain 2[24]. Mutant *BRAF* also increased motility through direct effects upon the cytoskeleton. In this instance, *BRAF* signaling directly regulates the expression of RND3/RhoE/Rho8, a protein that mediates the crosstalk between the RAF/MEK/ERK and Rho/Rock/LIMK/Cofilin pathways[25,26]. Depletion of RND3 using either siRNA or following BRAF/MEK inhibition led to an increase in focal adhesion stability and an inhibition of melanoma cell invasion in 3D cell culture models[25].

There is also evidence that oncogenic *BRAF* regulates the interaction of melanoma cells with the host microenvironment, particularly cells of the immune system. Inhibition of BRAF or MEK signaling in melanoma cells upregulates the expression of highly immunogenic differentiation antigens, leading to enhanced T-cell recognition[27–29]. The apparent ability of BRAF/MEK inhibitors to enhance the immune cell recognition of melanoma may offer a rationale for the future combination of BRAF inhibitors with immunotherapies such as ipilimumab[30].

The preclinical development of BRAF, MEK and ERK inhibitors in melanoma

At the time of the initial discovery of activating *BRAF* mutations in melanoma, selective BRAF kinase inhibitors had not been developed. One drug that was available at the time was the multi-kinase inhibitor sorafenib (Nexxavar ®)[31]. Although this was primarily a CRAF inhibitor, it did have some activity against mutant *BRAF* and was enthusiastically embraced as the first BRAF specific agent. Despite some activity in cell culture and the ability to stabilize disease in human melanoma xenograft models, sorafenib was ultimately found to have little activity in human melanoma patients, even when selected for their *BRAF* mutational status[32,33].

The latest generation of BRAF inhibitors offers a significant improvement over sorafenib in terms of potency against mutant *BRAF* and far fewer off-target effects. Compounds that have been evaluated preclinically include AZ628, XL281, GDC-0879, SB590885, GSK2118436 (GSK2118436) and vemurafenib (PLX4032, and its analogue PLX4720)[34–38] (Figure 2). Of these, vemurafenib and GSK2118436 have been the most extensively studied both preclinically and clinically. Vemurafenib is an ATP-competitive RAF inhibitor (WT *BRAF* IC_{50}, 100nM; mutant *BRAF* IC_{50}, 31 nM; CRAF IC_{50}, 48 nM) that selectively inhibits the phospho-ERK expression and growth of melanoma cell lines harboring *BRAF* V600E and V600K mutations[35,39]. In cell line models, vemurafenib induced both a G1-phase cell cycle arrest and an apoptotic response that was well correlated with increased BIM expression as well as the regression of established human *BRAF* V600E mutant melanoma xenografts[40,41].

One unexpected finding was that small molecule BRAF inhibitors activated MAPK signaling pathway in melanoma cells that were *BRAF* wild-type[42–45]. *In vitro*, the paradoxical activation of MAPK signaling arose in *BRAF* wild-type cell lines that harbored either an oncogenic *Ras* mutation or had upstream constitutive RTK activity (such as in HER2)[42,44,46]. The paradoxical activation of Raf that occurred following BRAF inhibition was found to increase proliferation and also enhanced the invasion of the melanoma cells through increased FAK activity as well as increasing the survival of *NRAS* mutated melanoma cell lines by increasing Mcl-1 expression[45]. Nearly all BRAF inhibitors evaluated to date, including sorafenib, vemurafenib, GSK2118436 and XL281 have been associated with the development of proliferative skin lesions most frequently identified as squamous cell carcinomas of the keratoacanthoma (KA) type[36,47,48]. These lesions, although rapidly growing, can be managed through surgical excision and do not recur. There is growing evidence that KAs arise as a result of the paradoxical activation of MAPK signaling following BRAF inhibition. Sequencing of a large cohort of KAs from patients that had spontaneous KAs, were immunosuppresed or were undergoing RAF inhibitor therapy demonstrated a significant enrichment for *Ras* mutations (21% vs 3%) in those individuals undergoing RAF inhibitor therapy[49,50]. These and other observations have prompted the development of a new generation of BRAF inhibitors, so-called "paradox breakers" (typified by PLX-Pb4) that apparently prevent the paradoxical activation of MAPK signaling. Although published data is currently lacking on these new drugs, it is hoped that their improved selectivity profile may prevent the development of KA and delay the time to resistance.

MEK and ERK inhibitors—A large body of preclinical work has demonstrated that virtually all melanomas have high constitutive activity in the MAPK pathway. The reasons for this are numerous, and can include activating *BRAF* and *NRAS* mutations, autocrine stimulation of growth factor receptors, such as c-MET and FGFR1[51], activation through αvβ3 integrin binding[52], and increased Notch1 signaling[53]. Most early studies of MEK inhibition in melanoma preceded the discovery of *BRAF* mutations and instead focused upon the role of MAPK in suppressing differentiation (an observation that is now proving critical for the development of BRAF/immunotherapy combinations)[54–56].

Preclinical studies have shown that inhibition of MEK in melanoma cells using inhibitors such as PD98059, PD0325901, CI-1040 and AZD6244 leads to a profound G1 phase cell cycle arrest[57–59] (Figure 2). These cytostatic effects are associated with the inhibition of cyclin D1 expression, increased p27[KIP1] expression and hypophosphorylation of the retinoblastoma protein[57,59]. In some sensitive *BRAF* mutant melanoma cell lines, MEK inhibition also induces apoptosis[60]. In general, the inhibition of MEK alone was not found to induce high levels of apoptosis and anti-tumor effects were enhanced following the dual inhibition of MEK and PI3K or by combining MEK inhibitors with chemotherapy drugs, such as paclitaxel[58,61] (Figure 2).

Another class of drugs targeting the MAPK pathway currently under development are the ERK inhibitors, AEZS-131 (Æterna Zentaris) and SCH772984 (Merck/Schering Plough). Although little published data is currently available on these compounds they seem pharmacologically distinct from MEK inhibitors and they may be of some future utility in overcoming vemurafenib resistance.

Clinical development of BRAF and MEK inhibitors

The first RAF inhibitor to be developed clinically in melanoma was the pan-kinase inhibitor sorafenib[31]. In early clinical trials, single agent sorafenib showed little efficacy and was associated with a median progression-free survival (PFS) of 3 months and only 1 response

out of 39 melanoma patients treated[62]. On the basis of phase II trials, where hints of sorafenib response were seen in combination with chemotherapy, large-scale phase III trials of sorafenib in combination with chemotherapy were initiated. The randomized studies showed variable results with low response rates and did not provide evidence that the clinical effects of sorafenib were mediated through RAF inhibition[33]. It now seems likely that any sorafenib activity observed in melanoma patients was independent of its effects upon BRAF and may instead have been a reflection of its off-target inhibitory effects on kinases such as VEGFR[32,63]. It is worth noting that the putative anti-angiogenic effects of sorafenib are the likely basis of the drug's efficacy in renal cell carcinoma[64].

The clinical failure of sorafenib led some to question whether oncogenic *BRAF* was really a good therapeutic target in melanoma. These concerns were quickly dispelled by the development of vemurafenib, a more potent and selective BRAF inhibitor, with fewer off-target effects than sorafenib[35]. In the phase I clinical trial, approximately 80% of *BRAF* V600E mutated melanoma patients treated at the recommended phase II dose of vemurafenib (960 mg, PO BID) responded to therapy. In all cases, responses were associated with inhibition of intratumoral phospho-ERK, reduction of Ki67 positivity by immunohistochemistry and the inhibition of glucose uptake as measured by FDG-PET[65]. An update of the phase I data, presented at the 2011 Society of Melanoma Research meeting, demonstrated the 1-year survival to be ~50% and the 2-year survival to be 38%, with a median overall survival of 13.8 months[66].

The phase II trial of vemurafenib (960mg BID) (**BR**af **I**n **M**elanoma: BRIM-2) enrolled 132 patients who had progressed on other therapies. In this cohort, there were 3 complete responses, 66 confirmed partial responses, and 39 patients with stable disease. Median OS has yet to be met and the medial PFS for this refractory population was 6.8 months[67]. The pivotal phase III trial (BRIM-3) randomized 675 untreated metastatic melanoma patients to either vemurafenib (960mg PO BID) or dacarbazine (the "standard of care" for metastatic melanoma). The study was terminated early in December 2010 because of a clear overall survival benefit seen to vemurafenib, with a hazard ration of 0.44 favoring vemurafenib over dacarbazine[48]. The overall response rate for vemurafenib was 48% and median PFS of 5.3 months as compared to 5% response rate and median PFS of 1.6 months for dacarbazine[48]. At six months, the Kaplan-Meier estimate of overall survival was 83% and 63% for vemurafenib and dacarbazine respectively. Correlative studies showed that a >90% reduction in intratumoral phospho-ERK was required for a clinical response to be seen[68]. Based upon the phase II trial data, vemurafenib was FDA-approved for unresectable metastatic *BRAF* mutant melanoma in late 2011.

BRAF V600E/K mutant melanoma patients treated with the BRAF inhibitor GSK2118436 showed similarly impressive responses[36]. A single-arm phase II study (BREAK-2) (n=92: 76 V600E mutant: 16 V600K mutant) showed objective response rates in the V600E cohort of 59% (95% CI 48–70%) with responses seen in 2 V600K patients. Median progression free survival was 27 and 20 weeks, respectively in the two mutation subsets. At this time GSK2118436 is currently undergoing phase III evaluation.

MEK inhibition—The identification of *BRAF* and *NRAS* mutations in significant numbers of melanoma patients and the observation that nearly all melanomas showed constitutive MAPK activity, led to the development of small molecule MEK inhibitors, such as PD0325901, selumetinib (AZD6244) and CI-1040 in unselected groups of melanoma patients[69–71] (Figure 2). The early studies on PD0325901 showed limited evidence of activity, with 2 out of 27 patients having partial responses and a further 5 patients having stable disease. In the phase I trial of AZD6244 one *BRAF* mutant melanoma patient was noted to have a complete response to AZD6244 persisting beyond 15 months[72]. However, a

subsequent large phase II trial of AZD6244 vs temozolomide in an unselected melanoma patient population showed no difference in PFS[73]. Overall these initial studies were disappointing and it was not clear whether these drugs were able to reliably inhibit the MAPK pathway at the doses used. The use of MEK inhibitors was further limited by diarrhea and visual disturbance with serious retinal vein thrombosis being reported in small numbers of patients.

Interest in the clinical development of MEK inhibitors in *BRAF* mutant melanoma was renewed by the development of GSK112012, an allosteric MEK inhibitor that potently inhibits MAPK signaling at clinically achievable doses[74,75]. At this time, GSK1120212 represents the most extensively studied MEK inhibitor in *BRAF* mutant melanoma and appears to have the best single agent activity of any MEK inhibitor evaluated thus far. In the phase II trial of 57 *BRAF* mutant melanoma patients previously treated with chemotherapy or immunotherapy, there were 2 complete responses, 17 partial responses, and 27 patients with stable disease, indicating an objective response rate of 33% and a disease control rate of 81%[76]. In the BRAF inhibitor naïve cohort, updated data showed the median duration of response to be 5.7 months, with a median PFS of 4 months[76]. Similar levels of response were seen in V600E or V600K mutated patients; however, there was minimal activity seen in the cohort of patients previously treated with a BRAF inhibitor. GSK1120212 is currently being evaluated in a Phase III trial (NCT01245062). It is worth noting that although GSK21120212 represents the best activity in its class, single-agent BRAF inhibition appears to show greater clinical efficacy.

Strategies to manage BRAF inhibitor resistance

Despite the impressive levels of tumor shrinkage observed in *BRAF* mutant melanoma patients treated with small molecule BRAF inhibitors, responses are typically short-lived (PFS: ~ 7 months) with resistance occurring in nearly every case[48,77]. The observation that ~50% of *BRAF* mutant melanoma patients on vemurafenib therapy did not meet the RECIST criteria for a response further suggests the existence of intrinsic BRAF inhibitor resistance[48]. Intrinsic resistance has been well documented in preclinical studies, with *BRAF* V600E mutant melanoma cell lines showing a wide range of IC_{50} values to vemurafenib and other BRAF inhibitors[38,78–80]. Although the reasons behind this are not yet clear, melanomas are known to harbor complex patterns of mutations and genomic amplifications, with alterations being reported in (among other things), *MITF, AKT3, COT, cyclin D1, CDK2, CDK4* and the retinoblastoma protein[79,81–85]. How these multiple factors interact to convey intrinsic resistance to BRAF inhibitors is currently the focus of intense investigation.

Intrinsic resistance to targeted therapy agents, such as trastuzumab in breast cancer, often results from increased basal levels of AKT signaling associated with the loss of PTEN expression/function[86]. In this instance, inhibition of EGFR is associated with impaired apoptosis and an overall worse response[86]. Emerging evidence suggests that impaired PTEN function may also, in part, mediate intrinsic vemurafenib resistance in melanoma[80,87] (Figure 3). This has been shown to result from increased AKT signaling when BRAF is inhibited, which limits the nuclear accumulation of FOXO3a, leading to a decrease in BIM-mediated apoptosis[87] (Figure 1). A recent clinical analysis of patients receiving the BRAF inhibitor GSK2118436 support these preclinical studies and show that *BRAF* mutant melanoma patients with a concurrent PTEN dysfunction exhibit lower response rates than *BRAF* mutant melanoma patients that retain PTEN function[88]. Similar findings were also reported in *BRAF* mutant melanoma cell lines treated with the MEK inhibitor AZD6244, where increased IGF-I mediated AKT signaling limited the apoptotic response[60]. In both of these cases, the IGF-I-mediated effects upon AKT signaling and intrinsic apoptosis resistance were overcome following treatment with a MEK + an AKT or mTORC1/2 inhibitor or a BRAF inhibitor + an AKT inhibitor[60,87] (Figure 3).

Inhibition of BRAF signaling in melanoma cell lines leads to the attenuation of MEK/ERK signaling, the inhibition of cyclin D1 expression and a G1-phase cell cycle arrest[38]. Work from our group identified a sub-set of *BRAF* mutant melanoma cell lines and tumor specimens with genomic amplification of cyclin D1[79]. Melanoma cell lines with cyclin D1 amplification in concert with a *BRAF* mutation showed intrinsic resistance to BRAF inhibition and continued to enter the cell cycle when oncogenic BRAF was inhibited[79]. Deregulation of the G1 cell cycle checkpoint, as a result of mutational inactivation of the retinoblastoma protein (RB1), in conjunction with PTEN loss (found in 2/19 of *BRAF* V600E melanoma cell lines) also conveyed intrinsic resistance to inhibitors of BRAF and MEK[80] (Figure 3).

Other recent studies identified protein kinase D3 (PRKD3) as being a potential mediator of intrinsic resistance to the RAF inhibitors RAF265 and vemurafenib[89] (Figure 3). In this instance, the siRNA knockdown of PRKD3 reduced the IC_{50} of both RAF265 and vemurafenib in multiple melanoma cell lines and prevented the reactivation of MAPK signaling following drug treatment[89]. In *BRAF* V600E mutant cell lines lacking PTEN expression, PRKD3 activity further contributed to resistance through the reactivation of PI3K/AKT signaling following RAF inhibition[89].

Acquired resistance to BRAF inhibitors occurs in the majority of patients treated[90]. Experimental studies suggest that minor populations of *BRAF* V600E-mutant melanoma cells exhibit drug tolerance and survive the initial treatment, with full resistance typically emerging after 3–6 months[38,91,92]. Unlike the resistance reported to other targeted therapies such as EGFR inhibitors in non-small cell lung cancer and imatinib resistance in gastrointestinal stromal tumors (GIST) and chronic myeloid leukemia (CML), resistance to BRAF inhibition is not associated with the acquisition of secondary (so called "gate-keeper") mutations in the kinase that prevent drug binding[93–95]. Although *in vitro* studies identified threonine-529 as the gate-keeper residue site in BRAF, a large scale sequencing analysis of the gene at exon 13 (where Thr-529 lies) did not identify this mutation in specimens from melanoma patients failing vemurafenib therapy[96,97]. Instead, preclinical studies where BRAF inhibitor resistance was generated *in vitro* showed that acquired resistance was mediated through a diverse array of mechanisms including constitutive signaling in receptor tyrosine kinases (IGF1R and PDGFR-β), increased expression of the MAP kinase family member COT (MAP3K8, TPL-2), acquisition of mutations in *NRAS* and *MEK1* and as the result of a BRAF truncations[37,83,97–100] (Figure 3). These observations support earlier pre-clinical studies showing that exogenously added growth factors and cytokines were able to rescue melanoma cells from cell death following siRNA-induced knockdown of BRAF[101,102]. The relative importance and frequency of each of these proposed resistance mechanisms in melanoma patients failing therapy are not currently clear and still require extensive clinical validation.

Although a number of potential vemurafenib resistance mechanisms have been reported, nearly all rely upon a common set of signaling pathways. Preclinical studies have already shown that reactivation of MAPK signaling is commonly associated with vemurafenib resistance and that combined MEK + BRAF inhibition is effective at abrogating the resistance mediated by MEK1 mutations, COT overexpression, BRAF truncation and acquired *Ras* mutations[38,83,99,100]. In contrast, resistance mediated through increased IGF1R signaling appears to be overcome by dual MEK + PI3K inhibition and resistance mediated through increased PDGFR-β signaling can be reversed through the targeting of the mTOR/PI3K/AKT pathway[98,103].

Clinical evaluation of BRAF inhibitor based combinations

The hypothesis that the recovery of MEK/ERK signaling underlies some of the resistance to BRAF inhibitors is currently being clinically validated in a phase I/II trial of the GlaxoSmithKline BRAF inhibitor GSK2118436 in combination with the MEK inhibitor GSK1120212 (NCT01072175) as well as a trial of vemurafenib in combination with MEK inhibitor GDC-0973 (NCT01271803). Early indications suggest these combinations may be effective, with the recent data presented at ASCO showing the GSK2118436 + GSK1120212 combination to be associated with an objective response rate of 77% (150mg GSK2118436, 1mg GSK1120212). Of interest, the combination was found to significantly reduce the incidence of keratoacanthoma (<1%, n=109), validating the role of rebound BRAF inhibitor mediated MEK/ERK signaling in the development of these tumors[74].

The phase I/II expansion cohort of the GSK2118436/GSK1120212 combination is also being evaluated in *BRAF* mutant melanoma patients who progressed on a prior BRAF inhibitor. In this study, 26 patients with metastatic melanoma who progressed on either GSK2118436 or vemurafenib alone were enrolled. Six of the patients had also previously received a MEK inhibitor. The median time since discontinuing prior BRAF inhibitor therapy was 1.1 months. The objective response rate was 19% but 62% of patients had some degree of tumor regression. The median progression-free survival was 3.6 months. Though most responses were short-lived, a subset of PR and SD patients (n=7) were still on therapy beyond 30 weeks[104]. In contrast, single agent GSK112012 had little efficacy in patients who progressed on prior BRAF inhibitor therapy[66].

Based upon preclinical observations demonstrating the dual requirement for the BRAF/ MEK and PI3K/AKT signaling pathways in melanoma progression, and the likely role of enhanced PI3K signaling in BRAF inhibitor resistance, clinical trials have been initiated to examine the PI3K/mTOR inhibitor BEZ235 in combination with the MEK inhibitor MEK162 (NCT01337765)[9,61,91,98,105]. This trial is currently enrolling patients who are *BRAF* mutant and those who are *NRAS* mutant.

The suggestion that BRAF targeted agents may enhance the effects of immunotherapy led to the initiation of a phase I/II clinical trial to evaluate the efficacy of vemurafenib in combination with the anti-CTLA-4 antibody ipilimumab (NCT01400451).

Future perspectives

The recent years have seen incredible progress in our management of advanced melanoma. If progress continues as we expect, a future can be envisaged in which rationally designed BRAF inhibitor-based drug combinations may be able to significantly extend the life span of *BRAF*-mutant melanoma patients. The possible development of successful BRAF inhibitor/ immune therapy based combinations offers the real possibility that very durable responses could be achieved. Although significant challenges remain as to the identification of good response biomarkers and strategies to overcome acquired drug resistance these remain exciting times for the melanoma community.

Acknowledgments

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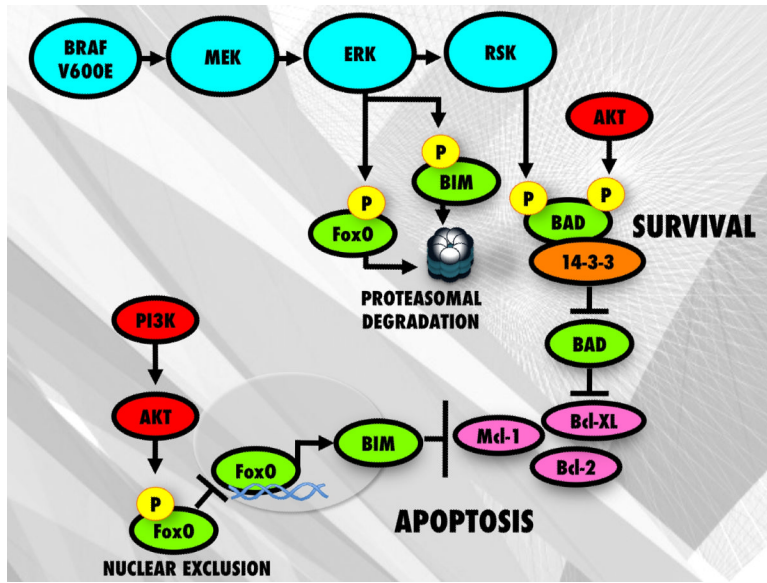
Figure 1. *BRAF* **V600E regulation of pro-apoptotic proteins promotes cell survival**
Inhibition of BRAF prevents proteasomal degradation of FOXO3a and BIM by blocking
MEK/ERK mediated phosphorylation of FOXO3a at Ser294, Ser344 and Ser425 as well as
phosphorylation of BIM at Ser69. However, in some melanomas with constitutive PI3K/
AKT activity, increased AKT signaling leads to phosphorylation and nuclear exclusion of
FOXO3a resulting in decreased expression of BIM. Both the MAPK pathway and AKT
pathways can also regulate the activity of BAD through direct phosphorylation.
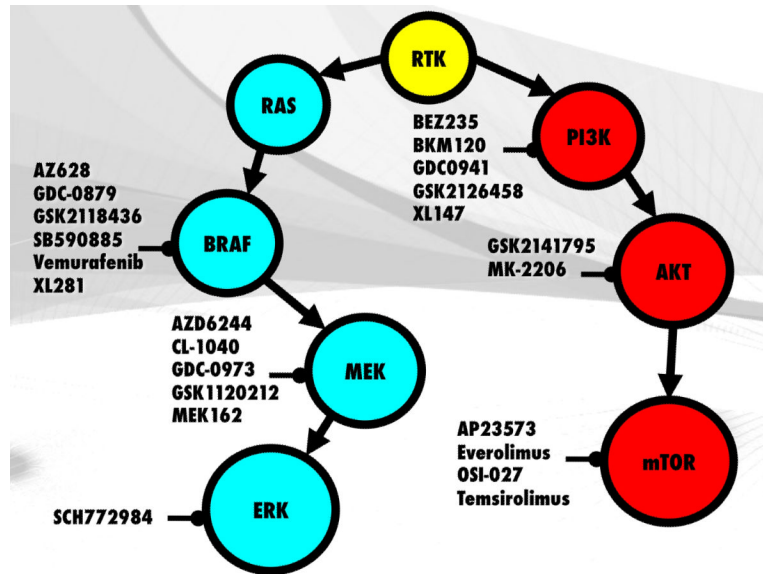
Figure 2.
Targeted kinase inhibitors of the MAPK and PI3K/AKT/mTOR pathways. Several compounds such as AZ628, dabrafenib, GDC-0879, SB90885 and XL281 are being evaluated for clinical use with vemurafenib being the only currently FDA approved BRAF inhibitor. Compounds that target the MAPK cascade such as the MEK inhibitor GSK1120212 and the ERK inhibitor SCH772984 as well as PI3K, AKT and mTOR inhibitors are also being developed as single and combined agents for the treatment of BRAF mutant melanomas.
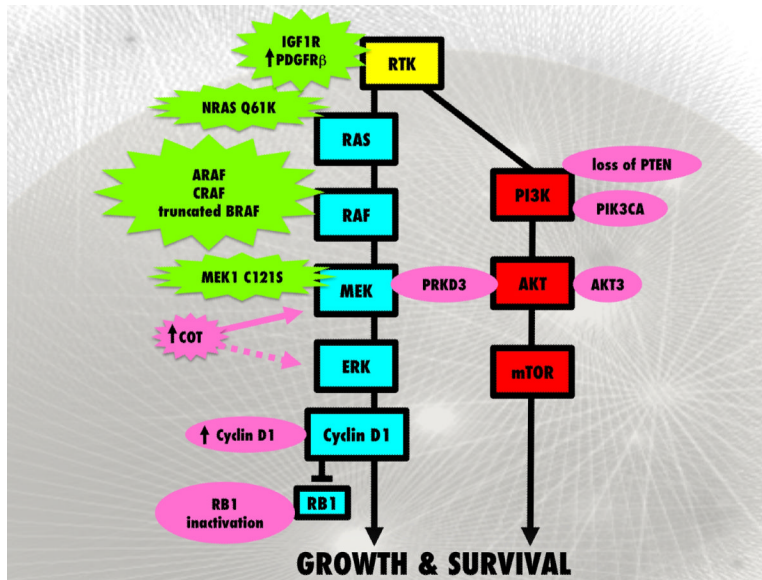
Figure 3. Known mechanisms of BRAF inhibitor resistance
Multiple acquired (shown in green) and intrinsic (shown in pink) mechanisms of BRAF inhibitor resistance have been identified. Chronic BRAF inhibition can lead to activation of the MAPK pathway through acquisition of secondary *NRAS* Q61K mutations, RAF isoform (ARAF>BRAF>CRAF) signal switching, truncation of BRAF to a BRAF inhibitor refractory form, *MEK1* C121S mutation as well as increased COT expression which has been linked to intrinsic as well as acquired resistance. RTK signaling via IGF1R and PDGFRb can also activate the MAPK and the PI3K/AKT pathways. Intrinsic mechanisms of resistance have been associated with amplification of cyclin D1 or RB1 inactivation allowing cells to continue through the cell cycle. Loss of PTEN and genetic alterations in *PIK3CA* and *AKT3* are associated with *de novo* resistance by increasing PI3K/AKT activity while PRKD3 activation can increase both MAPK and PI3K/AKT signaling.