Chapter 6
Parallel and Serial Blockade Strategies in BRAF-Mutant Melanoma

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Abstract Activating mutations in *BRAF* are the most common somatic aberration in cutaneous melanomas. These mutations result in constitutive activation of BRAF's catalytic activity and its downstream effectors in the RAS-RAF-MEK-ERK signaling pathway. Both selective BRAF and MEK inhibitors have demonstrated high clinical response rates in metastatic melanoma patients with activating *BRAF* mutations. These successes have illustrated several keys to the successful development of targeted therapies, and the potential for personalized therapeutic strategies for cancer. However, the ultimate clinical benefit of BRAF and MEK inhibitors has been limited by both de novo and secondary resistance mechanisms. Initial preclinical and clinical studies support that these resistance mechanisms may broadly be characterized as those that result in (1) re-activation of the RAS-RAF-MEK-ERK signaling pathway, or (2) activation of other pro-survival mediators. These findings are now leading to the development of new combinatorial approaches that involve serial and/or parallel blockade strategies in order to overcome resistance mechanisms, and ultimately to improve outcomes in melanoma patients with activating *BRAF* mutations. Further, these concepts are also being explored and tested in melanoma patients with other oncogenic mutations.

Keywords BRAF · Mutation · Amplification · Splicing · Targeted therapy · Resistance · MEK · NRAS · PI3K · AKT · mTOR · IGF1R · Immunotherapy · Combinatorial approaches

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6.1 Introduction

Melanoma is the most aggressive of the common forms of skin cancer. Although melanoma represents only ~5% of the skin cancers that are diagnosed each year, it is responsible for more than 70% of skin cancer-related deaths. While the incidence of many cancers has declined over the last few decades, the number of new cases of melanoma diagnosed every year continues to rise. Overall, the annual incidence of melanoma has increased over 600% since 1950. Unfortunately, many of the patients who are diagnosed with melanoma, and who ultimately succumb to the disease, are young, particularly women. Thus, melanoma has one of the highest life-years lost per cancer-related death among all malignancies. For these many reasons, melanoma is a significant disease which is likely to become an increasingly important public health issue in the future if current trends are not reversed [1].

Multiple treatment modalities are utilized in the care of melanoma patients. Surgery is the mainstay of treatment for patients with both clinically localized (i.e. cutaneous primary tumor) and regionally metastatic (i.e. regional lymph nodes or in-transit disease) disease, and may also be utilized for palliation in patients with distant metastases. Radiation therapy has a clear role for palliation of painful metastases, but its benefits in earlier, potentially curable stages of disease are less clear [2]. Systemic therapies are used in some patients to reduce the risk of relapse after surgical treatment of regional metastases [3], and they are generally the primary treatment modality for patients with distant metastases or unresectable regional tumors.

Although cytotoxic chemotherapies represent the backbone of systemic therapy for most cancers, historically these agents have demonstrated minimal benefit in patients with metastatic melanoma [4]. For example, dacarbazine (DTIC) was approved for use in metastatic melanoma in the mid-1970s despite achieving clinical responses in ≤10% of patients and having no demonstrated (or appreciable) impact on median progression-free (PFS) or overall survival (OS). Combining chemotherapy agents together in various regimens resulted in increased toxicity, but no proven impact on survival [1]. With these disappointing results, other therapeutic strategies have been investigated extensively in melanoma. Much of this effort has focused on the development of agents that stimulate the immune system to attack or control the cancer, which as a class have been termed immunotherapies. High-dose bolus interleukin-2 (HD IL-2) therapy was the first such agent to gain approval in patients with metastatic melanoma, in 1998. Non-randomized studies of metastatic melanoma patients treated with HD IL-2 demonstrated that this therapy was able to achieve durable (>10 year) disease control in metastatic melanoma patients, leading to it regulatory approval [5, 6]. However, this was only achieved in the patients who had complete responses to treatment, which only occurred in ~5% of patients. Overall, only 15% of patients achieved even transient clinical responses. Further, HD IL-2 therapy is extremely toxic, requiring ICU-level care to manage the many side effects of the treatment, and resulting in treatment related deaths in ~1% of

patients in early phase clinical trials. More recently, a number of new strategies and agents have been identified to stimulate anti-tumor immune responses. Most notably, ipilimumab, an antibody that blocks the inhibitory CTLA-4 receptor on the surface of T cells, was granted regulatory approval for patients with metastatic melanoma in 2011. While ipilimumab has a moderate clinical response rate of only ~10%, in randomized clinical trials treatment with this agent resulted in statistically significant improvements in PFS and OS compared to controls, and a three year survival rate of ~25% [7, 8]. In contrast to HD IL-2, ipilimumab has very few acute side effects and can be given in the outpatient setting. However, ipilimumab can produce significant autoimmune toxicities in some patients, including colitis, hepatitis, and endocrinopathies.

A relatively new systemic therapy modality to be explored in melanoma is targeted therapy. Conceptually, targeted therapies inhibit the molecules and/or pathways that are specifically dysregulated in cancer cells. Targeted therapies have demonstrated efficacy in a number of diseases, including those that are generally refractory to chemotherapy [9]. One of the earliest examples of the potential of targeted therapy was the development of imatinib for chronic myelogenous leukemia (CML). Almost all CML cells are characterized genetically by a translocation event between chromosomes 9 and 22, resulting in the characteristic Philadelphia chromosome that is the hallmark of this disease. This genetic event produces a novel fusion protein (BCR-ALB) that includes the kinase domain of the *ABL* gene. Imatinib, a small molecule inhibitor of ABL and other kinases, produced marked improvements in clinical outcomes even in very early phase clinical trials in CML, and rapidly became the standard of care of patients with this disease [10]. Targeted therapies have also become the standard of care for specific, molecularly-defined subpopulations of other cancers, including breast cancers with amplification of the *HER2/neu* gene (trastuzumab) and lung cancers with *EGFR* mutations (erlotinib) [11–14]. While targeted therapies have proven clinical benefit in these populations, efficacy is frequently limited by the rapid development of resistance. An improved understanding of the mechanisms of resistance is now leading to the development of new inhibitors and/or combinatorial strategies that aim to achieve a greater degree or duration of cancer control across multiple tumor types.

Perhaps more than any other cancer, the recent history of the development of targeted therapy for melanoma demonstrates both the promise and challenges of this therapeutic strategy. Specifically, the development of targeted therapies for melanomas with activating mutations in the *BRAF* gene has illustrated a number of key factors in this area of research. Further, both clinical and preclinical studies have now set in motion the development of various combinatorial strategies for this disease. The following is a summary of the foundation that had led to this new era of combinatorial therapies, and the rationale behind several of the leading combinations that are being pursued.

6.2 Molecular Biology of Melanoma

The sentinel event in the development of targeted therapy for melanoma was the discovery of point mutations in the *BRAF* gene [15]. These mutations were identified as part of a screen for mutations in the genes that encode the RAF kinases, which are part of the RAS-RAF-MEK-ERK signaling cascade (Fig. 6.1). This initial screen of cell lines and tumors identified recurrent point mutations in exon 15 of the *BRAF* gene, most frequently in the melanomas that were included in the study, but also in colorectal, primary brain, lung, liver, ovarian, and other cancer types. Subsequent studies have demonstrated that more than 90 % of the *BRAF* mutations that are detected in melanoma occur in exon 15 and result in substitutions for the valine at the 600 position (V600) [16]. The most common mutation results in substitution of a glutamic acid (V600E), which in multiple series has been shown to represent ≥ 70 % of the detected *BRAF* mutations [17, 18]. The catalytic activity of the BRAF V600E mutant protein is increased more than 400-fold in comparison to the wild-type BRAF protein and results in constitutive activation of MEK and ERK. Other substitutions at the V600 site, including V600K and V600D, also markedly (more than 100–200-fold) increase the catalytic activity of BRAF. A variety of other

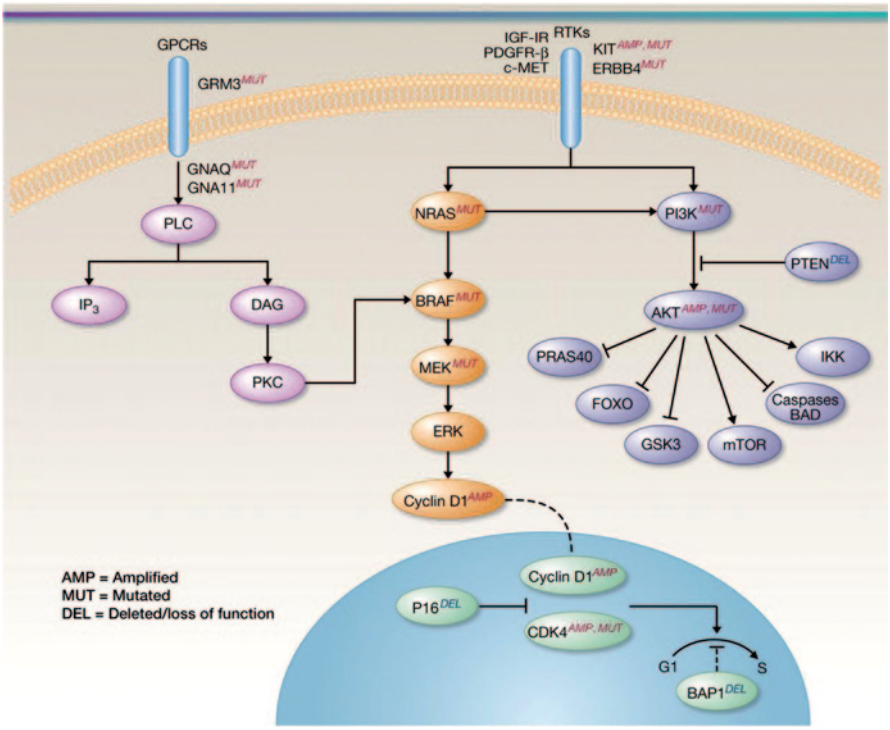

Fig. 6.1 Frequent somatic mutations in signaling pathways in melanoma. (Used with permission from [22])

rare point mutations in BRAF have also been detected, both in exon 15 (i.e. K601E, L597V) and exon 11 (i.e. G469E, G464E). Interestingly, these mutations are quite variable in their effects on the catalytic activity of BRAF, with some mutations actually resulting in decreased kinase activity (i.e. G466E, D594V, and G596R) [19]. However, expression of essentially any of these mutations results in increased activation of MEK and ERK, as the kinase-inactivating mutations promote the formation of BRAF-CRAF heterodimers that activate the pathway through CRAF's catalytic activity [20].

Meta-analyses of large cohorts of melanoma clinical samples have demonstrated that substitutions of the V600 residue of BRAF occur in 40–50% of cutaneous melanomas [16] (Table 6.1). These mutations are most frequent in cutaneous melanomas arising in areas with intermittent sun exposure, but are less common in tumors that arise in areas of chronic sun exposure and have histologic evidence of chronic sun damage (CSD) [21, 22]. The mutations are less prevalent (10–15%) in acral melanomas, which arise on the relatively sun-protected palms of the hands, soles of the feet, and nailbeds. Mucosal melanomas, which arise from mucosal surfaces throughout the body, have a *BRAF* mutation rate of <5%. Finally, *BRAF* mutations have not been detected in uveal melanomas that arise from melanocytes in the eye.

Activating mutations in *NRAS,* which also activate the RAS-RAF-MEK-ERK signaling pathway, are the second most common somatic activating mutations detected in melanoma. These mutations occur in 15–20% of cutaneous melanomas, most commonly resulting in substitutions at the Q61 residue of exon 2 (~80% of mutations) or the G12/13 residues of exon 1 (~20%) [16]. *NRAS* mutations are also detected in acral and mucosal melanomas, but are not found in uveal melanomas (Table 6.1). In treatment-naïve patients, hotspot *NRAS* mutations and *BRAF V600* mutations are essentially mutually exclusive, with both mutations found in less than 1% of tumors [17]. However, *NRAS* mutations are frequently detected in melano-

Table 6.1 Prevalence and pattern of common somatic mutations in different melanoma subtypes. "CSD", chronic sun damaged. "–", insignificant number reported. "?", not yet reported. (Adapted with permission from [22])

	Mutations				
	BRAF	NRAS	KIT	GNαQ/11	BAP1
Cutaneous (Non-CSD)	45%	15–20%	~1%	–	?
Cutaneous (CSD)	5–30%	10–15%	2–17%	–	?
Acral	10–15%	10–15%	15–20%	–	?
Mucosal	5%	5–10%	15–20%	–	?
Uveal	–	–	–	80%	50% (85% of monosomy 3)
Melanoma from an Unknown Primary	50%	20%	–	–	–

mas with non-V600 BRAF mutations, particularly those that fail to increase the catalytic activity of BRAF [20]. Similar to NRAS, strong genetic interaction has also been identified for loss of function mutations of the *PTEN* tumor suppressor [23]. PTEN is a phosphatase that dephosphorylates phospho-lipids in the cell membrane, thereby antagonizing signaling by the oncogenic lipid kinase PI3K. Loss of PTEN results in constitutive signaling through the PI3K-AKT pathway. A number of analyses have demonstrated that loss of function and/or expression of PTEN in melanomas are mutually exclusive with the presence of *NRAS* mutations [24–26]. In contrast, PTEN can occur in melanomas with activating BRAF mutations, and is detected in 20–30% of BRAF V600-mutant melanomas.

Focused sequencing studies have identified a number of other somatic changes in oncogenes in melanoma in, or downstream of, the canonical RAS-RAF-MEK-ERK and PI3K-AKT pathway, such as rare activating point mutations in *AKT1, AKT3, MEK1,* and amplifications of cyclin D1 [27–29] (Fig. 6.1). In addition, deletions and inactivating mutations of the *CDKN2A* gene that cause loss of expression/function of the P16 protein are germline mutations in many cases of familial melanoma, and may also occur somatically [30]. Activating mutations and amplifications of the *CDK4* gene are also detected in melanomas as germline or somatic events [31]. In addition to these events in cutaneous tumors, studies have revealed a number of mutations in other melanoma subtypes. Somatic mutations and gene amplifications of the *KIT* gene on chromosome 4 have been identified as frequent events (10–30%) in acral and mucosal melanomas [32]. Some studies have also suggested that these mutations are also common in cutaneous melanomas with evidence of chronic sun damage (CSD), but this has not been observed in other studies [33]. Molecular characterization of uveal melanomas demonstrated a lack of *BRAF, NRAS,* or *KIT* mutations in these tumors, but loss of expression of PTEN has been observed [34, 35]. Uveal melanomas instead have a high prevalence of activating point mutations in the *GNαQ* (35%) and *GNα11* (45) genes, which encode regulatory subunits of G-protein coupled receptors [36–38]. As these mutations are mutually exclusive, altogether they are present in ~80% of uveal melanomas, and preclinical studies suggest that they can cause activation of multiple signaling pathways. Approximately 80% of uveal melanomas that have monosomy 3, which correlates with poor prognosis, have inactivating mutations of the *BAP1* gene, which is located at 3p21 [39]. Germline mutations in *BAP1* have also been identified in families with an increased risk of developing uveal melanoma [40, 41].

Recently, broad sequencing efforts that characterize the entire exome or genome have been initiated melanoma [42–45]. These studies have demonstrated that cutaneous melanomas have an extremely high somatic mutation rate. The majority of the observed somatic mutations were C → T or G → A transitions, which are associated with DNA damage from ultraviolet radiation (UVR) [46]. This data is consistent with multiple functional and epidemiological studies implicating UVR in the development of melanoma [47]. These broad sequencing studies have demonstrated the molecular complexity and heterogeneity of melanomas (Fig. 6.2) [43]. The studies have identified many additional somatic events that occur in melanomas with activating *BRAF* or *NRAS* mutations, as well as candidate drivers in melanomas that

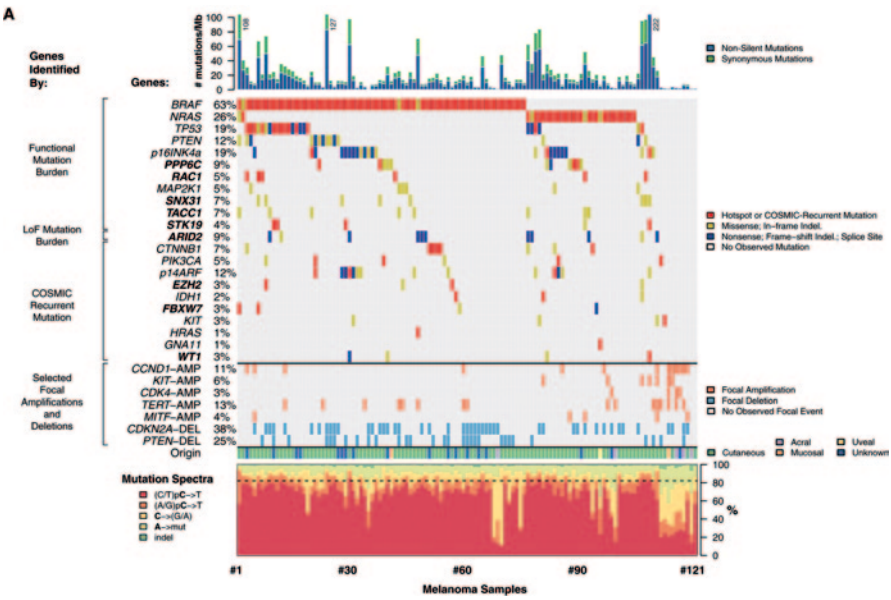

Fig 6.2 Pattern of novel and known somatic alterations in a cohort of 121 melanomas. (Adapted with permission from [43])

do not have a hotspot mutation in either of those genes. While these studies have already provided significant insight into the molecular pathogenesis of melanoma, it has also illuminated that a critical challenge to researchers will be to determine which mutations are clinically significant. In addition to being therapeutic targets, mutations may have clinical utility if they add to risk prediction models that are used to guide the selection of treatments for patients, or to inform the appropriate design of clinical trials [17, 18, 48]. While the large number of alterations observed in melanoma makes this overall appear to be a daunting challenge, the clinical experience with BRAF V600-mutant melanomas has already demonstrated the tremendous clinical impact such findings can have.

6.3 Development of BRAF Inhibitors

Early preclinical studies demonstrated that inhibition of BRAF in melanoma cell lines and xenografts with V600 BRAF mutations significantly slowed growth both in vitro and in vivo [49–51]. Based on this promising data, the effects of BRAF inhibition were tested in melanoma patients in clinical trials. Initial clinical trials mainly were performed with sorafenib. Sorafenib is a small molecule inhibitor of many kinases, including BRAF, although it actually binds to other targets (i.e. CRAF) with greater affinity. The first clinical trial of sorafenib in metastatic melanoma

patients demonstrated that less than 5% of patients achieved a clinical response with this agent [52]. Another trial in which patients were treated with paclitaxel and carboplatin, and then were randomized to receive sorafenib or a placebo, again demonstrated that sorafenib had minimal impact on clinical response rates or progression-free survival (PFS) [53].

While these results were disappointing, a second wave of testing was precipitated by the development of drugs that were designed to be highly selective inhibitors of BRAF- and specifically of the BRAF V600E mutant protein encoded by the most common mutation of this gene. The first such agent to undergo testing was vemurafenib (also called PLX4032) [54]. Preclinical studies demonstrated that vemurafenib potently inhibited the MAPK signaling pathway, growth, and survival of BRAF V600-mutant human melanoma cell lines, but almost no effect was seen in cell lines without such a mutation [55]. Treatment of xenografts of these cell lines in mouse models demonstrated that the vemurafenib treatment caused tumor regression. This impressive activity accurately predicted the results seen in patients. In the phase I clinical trial of vemurafenib, approximately 80% of the patients with BRAF V600E-metastatic melanoma had significant tumor shrinkage; in contrast, none of the 5 patients who did not have this mutation responded [56]. Subsequent preclinical studies in melanoma and other cancers by multiple groups found that treatment of cancer cells that did not have a BRAF V600 mutation, and particularly those with activation of RAS proteins, with vemurafenib and other compounds in this class caused increased tumor growth in vitro and in vivo [20, 57–59]. These studies showed that selective inhibitors of the BRAF V600-mutant protein actually caused increased activation of the MAPK pathway in these cell lines, as measured by increased phosphorylation of activation-specific sites on both MEK and ERK. This effect appears to be due to inhibitor-induced changes in the structure of the wild-type BRAF protein which results in a conformation that facilitates the formation of heterodimers with CRAF proteins. These BRAF-CRAF heterodimers activate MEK and ERK, and subsequently increase the growth of the tumor cells. Interestingly, this paradoxical activation of the MAPK pathway appears to be largely responsible for an interesting toxicity seen with vemurafenib: the development of cutaneous squamous cell carcinomas (SCCs) and/or keratoacanthomas (KAs). These lesions are observed in 20–25% of patients treated with vemurafenib, and are generally treated successfully with surgery [56]. Molecular analyses demonstrated that these lesions frequently have mutations in *RAS* genes, and they demonstrate increased MAPK pathway activation following treatment with the mutant-selective BRAF inhibitors [60, 61]. This mechanism was recapitulated in animal models. Importantly, these studies demonstrated that adding a MEK inhibitor to the mutant-selective BRAF inhibitor blocked the formation of these hyperproliferative cutaneous lesions [60].

In addition to the critical importance of selecting patients with BRAF V600 mutations for treatment with vemurafenib, the phase I trial also demonstrated the specific relevance of MAPK pathway inhibition to the observed clinical benefit. A series of patients enrolled in the phase I trial underwent biopsies of their tumors before the start of treatment, and after 1 to 2 weeks of therapy. Analysis of P-ERK expression by immunohistochemistry (IHC) demonstrated that variable degrees

of MAPK pathway inhibition were achieved in these patients with vemurafenib treatment. When the changes in P-ERK were compared to the maximal changes in tumor size, a nearly linear relationship between these two factors was observed [62]. Greater inhibition of the pathway correlated with greater inhibition of tumor growth. This finding reinforced the importance of this pathway that was implied by the high prevalence of mutations observed in melanoma.

Subsequent clinical testing of vemurafenib was limited to patients with metastatic melanoma with V600E BRAF mutations. In the pivotal BRIM-3 phase 3 trial, such patients were randomized to treatment with vemurafenib or dacarbazine [63]. This trial was halted at its first analysis, and it was the shortest phase III clinical trial ever conducted in oncology. Treatment with vemurafenib produced significant improvements in response rate (48 versus 5%, $p<0.001$), PFS (median 5.3 versus 1.6 months, Hazard ratio [HR] 0.26, $p<0.001$), and OS (6 month OS 84 versus 64%, HR 0.37, $p<0.001$). Based on this data vemurafenib received regulatory approval for the treatment of metastatic melanoma patients with BRAF V600E mutations in 2011.

Dabrafenib is a structurally unrelated small molecule that also is a highly potent and selective inhibitor of V600-mutant BRAF proteins [64, 65]. In a randomized phase III trial comparing dabrafenib to dacarbazine in metastatic melanoma patients with BRAF V600E mutations, dabrafenib treatment resulted in significant improvements in response rate (50 versus 6%) and PFS (5.1 versus 2.7 months, HR 0.30, $p<0.0001$) [66]. The effects on OS did not reach statistical significance (HR 0.61, 95% confidence interval [CI] 0.25–1.48). However, in this trial patients who progressed on dacarbazine were allowed to cross-over to the dabrafenib treatment, which was not allowed in the BRIM-3 trial of vemurafenib. A third mutant-selective BRAF inhibitor, LGX818, is currently in early phase clinical testing[1].

The relatively short time that elapsed from the discovery of activating BRAF mutations to the regulatory approval of vemurafenib and dabrafenib stands as a powerful example of the speed and potential impact of genomics and translational research. It is clear that the selective BRAF inhibitors have delivered tremendous clinical benefit to patients with this highly aggressive disease. Indeed, symptomatic improvement is often observed within days of starting treatment. Frustratingly, however, the clinical benefit of the BRAF inhibitors is variable, and often short-lived. For example, in the BRIM-3 trial, only ~3% of patients had disease progression as their best response, reinforcing that almost all patients experienced some degree of disease control. However, only 2 out of 219 patients achieved a complete response, and ~50% of patients achieved only minor clinical responses (<30% reduction in tumor size) [63]. This tremendous variability in the degree of tumor response likely reflects pre-existing heterogeneity among patients and/or tumor cells with activating BRAF mutations. Furthermore, the median duration of the responses with the BRAF inhibitors has generally been only 5–7 months in the various clinical trials with vemurafenib and dabrafenib [56, 63, 66, 67]. Approximately 90% of patients develop resistance within 1 year of starting treatment. This resumption of growth

[1] www.clinicaltrials.gov.

after initial responsiveness to the BRAF inhibitors reflects the development of acquired, also called secondary, resistance.

Research has now identified a variety of mechanisms that may mediate resistance to the selective BRAF inhibitors. In general terms, these mechanisms either (1) cause re-activation of MAPK pathway effectors, or (2) result in activation of other pro-survival pathways. Similar to the selective benefit of vemurafenib and dabrafenib in patients with V600 BRAF mutations, these findings support the rationale to develop personalized approaches that will overcome these various mechanisms.

6.4 Rationale for Dual Inhibition of the MAPK Pathway

The strong correlation between MAPK pathway inhibition and clinical benefit observed in the clinical development of the selective BRAF inhibitors led to the hypothesis that resistance could be due to reactivation of signaling by the pathway. Due to the highly selective effects of the BRAF inhibitors in melanoma cells with V600 BRAF mutations, and the paradoxical pathway activation and growth observed in cells without these mutations, one explanation for the emergence of resistance could be the selective depletion of BRAF-mutant cells from molecularly heterogeneous tumors. Indeed, some studies have suggested that different regions of individual tumors vary in the relative frequency of cells with and without BRAF mutations [68]. However, sequencing analyses of melanoma samples collected at the time of resistance in multiple studies have demonstrated in all cases the continued presence of the same activating BRAF mutation that was present before the start of therapy [69, 70]. Similar results were also observed in cell lines that were selected in vitro for secondary resistance through chronic exposure to increasing doses of the BRAF inhibitors.

A second potential mechanism that could potentially cause resistance to the BRAF inhibitors would be the acquisition of secondary mutations in the BRAF gene. Secondary mutations in drug targets are a common finding in CML and gastrointestinal stromal tumors (GISTs) that have developed resistance to imatinib. Preclinical studies demonstrated that artificially introducing mutations at the Thr529 gatekeeper residue of BRAF could negate the inhibitory effects of vemurafenib and other selective BRAF inhibitors in melanoma cell lines [71]. However, despite this demonstration, and the experience with other targeted therapies, to date no secondary mutations in the BRAF gene have been identified in resistant melanoma tumors or cell lines [69].

While new mutations in BRAF have not been identified as a mechanism of resistance, two other alterations have: copy number gain and alternative splicing. Copy number gain of the mutant BRAF allele was identified in 4 of 20 (20%) progression samples by whole exome sequencing, with corresponding increased BRAF protein expression [72]. Resistance in cell lines with BRAF copy number gain could be overcome by treating the cells with increased doses of the selective

BRAF inhibitors, suggesting a therapeutic strategy for patients with resistance due to this mechanism. However, this strategy will likely not be effective in patients with resistance due to aberrant splicing of BRAF. This phenomenon was identified in 6 of 19 (32%) progression samples from patients, as well as in several cell lines selected for resistance, which demonstrated expression of a smaller (61 kDa) form of the BRAF protein [73]. This truncated form of the protein efficiently forms heterodimers with CRAF, which subsequently activates MEK and ERK. This interaction between CRAF and the truncated BRAF was not prevented by treatment with increased doses of the selective inhibitors of BRAF. However, the continued dependence on MAPK pathway signaling was demonstrated by the fact that the cells remained sensitive to MEK inhibitors. The utilization of heterodimers by BRAF with other RAF isoforms at the time of resistance was also identified by another group of investigators, although the mechanism underlying the switch to this capability was not identified [74]. Those studies demonstrated that treatment of the studied resistant cell lines with MEK inhibitors was able to block activation of the pathway and induce growth inhibition. However, in contrast to the parental (sensitive) cells from which the resistant clones were selected, MAPK pathway inhibition alone was not sufficient to induce apoptosis, suggesting the potential for additional aberrations to be driving resistance concurrently.

In addition to alterations in BRAF, alterations in other members of the MAPK pathway produce reactivation of MEK and ERK signaling in spite of continued exposure to BRAF inhibitors. As mentioned previously, co-occurrence of BRAF V600E and activating NRAS mutations is detected in less than 1% of newly diagnosed melanomas. However, this overlap is more common after exposure to selective BRAF inhibitors. The presence of activating NRAS mutations was initially described in 2 progressing tumors derived from the same patient; interestingly, the tumors actually had different NRAS mutations (Q61K and Q61R), implying that they had arisen from independent clones [69]. NRAS mutations were also identified in 4 of 19 (21%) progressing lesions in another study, and were mutually exclusive with aberrant splicing of BRAF [73]. In vitro studies demonstrated that the presence of a concurrent NRAS mutation results in re-activation of ERK via CRAF and remains sensitive to MEK inhibitors. Whole exome sequencing of a single patient with acquired resistance to a BRAF inhibitor identified acquisition of a somatic mutation that resulted in a C121S substitution in MEK1 as a cause of resistance [75]. A subsequent sequencing analysis of *MEK* in clinical samples obtained before the start of treatment with vemurafenib and at the time of progression identified several mutations in the gene. Interestingly, some of the mutations (i.e. P124L substitution) were identified in the pre-treatment samples in patients who achieved clinical responses, suggesting that they were not sufficient to cause resistance. However, other mutations (i.e. Q56P) were identified only in progressing lesions, and thus likely causative of disease progression [70]. This heterogeneity implies that additional studies will be needed over time to classify the functionality and clinical significance of various MEK mutations [76]. Finally, overexpression of COT, a serine-threonine kinase that is capable of activating downstream components of the MAPK pathway, was observed following BRAF inhibition in 2 of 3 patients

samples obtained early in their treatment with vemurafenib, and in 1 patient was highest at the time of disease progression [77]. While in vitro studies suggested that COT may be able to phosphorylate ERK directly, BRAF inhibitor-resistant cells with enforced COT expression remained sensitive to MEK inhibitors.

The identification of multiple molecular aberrations that cause reactivation of MAPK pathway signaling supports the rationale to target this pathway at multiple levels (Fig. 6.3) [78]. Analysis of tumor biopsies obtained after 2 weeks of treatment in the phase II clinical trial of vemurafenib demonstrated that patients who did not achieve clinical responses had significantly less inhibition of ERK activation than patients who responded [70]. This demonstration of early, incomplete inhibition of the pathway in some patients suggested that combined inhibition may not only be an effective strategy to use after acquired resistance develops, but also potentially as a way to improve the magnitude, and hopefully duration, of the initial responses to therapy. These hypotheses are now supported by the clinical experience with combinatorial therapy with BRAF and MEK inhibitors.

Trametinib is an orally available potent inhibitor of MEK1/2 [79]. Clinical testing has demonstrated that trametinib has activity as a single agent in metastatic melanoma patients with BRAF V600 mutations who have not previously been treated with BRAF inhibitors. In a randomized phase III trial of trametinib versus chemotherapy that allowed cross-over at the time of progression, trametinib treatment

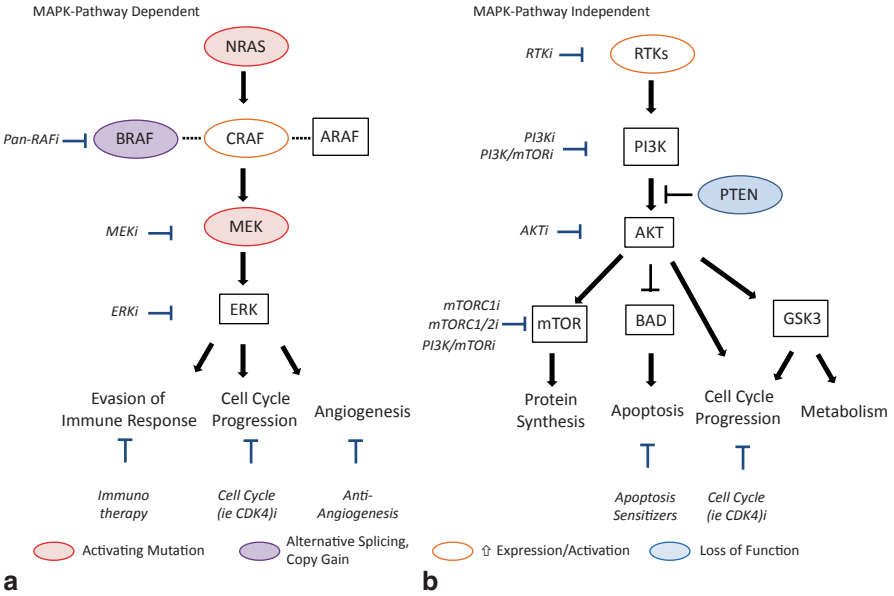

Fig 6.3 Resistance mechanisms and combinatorial strategies for BRAF-mutant melanomas. Schema of described mechanisms of resistance to selective inhibitors of mutant BRAF in melanoma. **a** MAPK-pathway dependent mechanisms. **b** MAPK-pathway independent mechanisms. Classes of agents that may be used to target components of the pathways are indicated to the side in each panel. (Used with permission from [78])

produced significant improvements in response rate (22 versus 8%, $p=0.01$), PFS (4.8 versus 1.5 months, HR 0.45, $p<0.0001$), and OS (6 month OS 81 versus 67%, HR 0.54, $p=0.01$) [80]. While the enthusiasm about these results were dampened specifically in the melanoma field in light of the parallel development and results of selective BRAF inhibitors, this trial represents the first positive phase III trial for a MEK inhibitor in any cancer type, thus confirming the potential for clinical utility for these agents. However, even more impressive results were observed when trametinib was combined with the selective BRAF inhibitor dabrafenib. A randomized phase II study was conducted in BRAF inhibitor-naïve metastatic melanoma patients with BRAF V600 mutations (V600E or V600K) [81]. All patients received the standard dose of vemurafenib (150 mg twice daily), and then were randomized to receive placebo, half-dose (1 mg per day; referred to as "150/1" treatment) or full-dose (2 mg per day; "150/2") trametinib after these combinations were demonstrated to be safe and well-tolerated. Consistent with preclinical studies implicating paradoxical activation of the MAPK pathway as the mechanism of cutaneous SCCs and KAs from BRAF inhibitor therapy, the incidence of these lesions was markedly reduced in patients who received MEK combination therapy (2% with 150/1, 7% with 150/2) compared to those who received dabrafenib alone (19%). Patients who received the combination were also less likely to develop rashes, although other toxicities (i.e. acneiform dermatitis, fevers/chills, nausea/vomiting, diarrhea, neutropenia) were more frequent. However, these toxicities were generally manageable with supportive care or interruption of treatment. More importantly, the combination demonstrated significant improvements in multiple clinical outcomes. The clinical response rates were 54% for dabrafenib monotherapy, 50% for the 150/1 combination, and 76% (including 9% complete responses) for the 150/2 combination. The median PFS was 5.8 months for dabrafenib monotherapy, 9.2 months for 150/1 (HR 0.56, $p=0.006$), and 9.4 months for 150/2 (HR 0.39, $p<0.001$). At 12 months only 9% of patients treated with dabrafenib alone remained progression-free, compared to 26% with 150/1 and 41% with 150/2.

Trametinib has also undergone early evaluation in patients who have progressed on BRAF inhibitors. Despite preclinical evidence that cells with acquired resistance to BRAF inhibitors often remain sensitive to MEK inhibition, to date these results have been relatively disappointing. Treatment with single agent trametinib in failed to result in a clinical response in 37 patients who had developed resistance to a selective BRAF inhibitor, although 2 of 3 patients who had stopped BRAF inhibitor therapy due to toxicities did respond [82]. The median PFS of the patients overall was only 1.8 months. Combined treatment (150/2) with dabrafenib and trametinib achieved clinical responses in 4 out of 21 (20%) patients who had previously progressed on a BRAF inhibitor, and in 1 of 5 (20%) patients who had progressed on a MEK inhibitor [83]. Although full interpretation of the results will require additional follow-up to allow for meaningful assessment of time-dependent outcomes, one implication of the results is that it may be more effective to continue BRAF inhibitors and add other agents to this therapy in patients who progress on BRAF inhibitors than to simply change them to different targeted agents. This finding, if confirmed, would be similar the clinical experience in HER2/neu-positive breast cancer patients following progression on trastuzumab.

While the slight improvement observed with the combination of dabrafenib and trametinib treatment compared to trametinib alone in the progressing patients is interesting, overall the relatively low activity has been disappointing in the face of the results observed in BRAF inhibitor naïve patients, and the multiple studies supporting reactivation of MEK at the time of resistance. The evidence of significant benefit in some patients, however, does suggest that it may be possible to predict which patients this regimen is effective in by comparison of the clinical outcomes to the underlying resistance mechanisms. Alternatively, early assessment of the regimen's ability to inhibit ERK activation may predict benefit. However, no data has been presented to date testing either possibility. In turn, while combined inhibition of BRAF and MEK as front-line therapy has been very impressive, it is clear that many patients are still developing resistance in a relatively short period of time, and it is unclear how many, if any, of the patients treated with the combination are achieving the durable disease control that is seen in some patients treated with immunotherapies.

Despite these limitations, the rapid advances in outcomes that have been achieved again demonstrate the dramatic potential clinical benefit for rational combinatorial treatment approaches. Additional testing is currently ongoing with other BRAF and MEK inhibitors to determine whether differences in pharmacological properties may result in greater efficacy. Alternative dosing regimens have also been proposed as another strategy to prevent or delay resistance in preclinical models, but there is no clinical data yet addressing this hypothesis [84]. Evaluation of inhibitors of other targets in the MAPK pathway, including ERK, is also ongoing [85]. However, multiple lines of evidence also suggest that strategies that combine MAPK pathway inhibition with targeting of other pathways may be an effective clinical strategy for some patients.

6.5 The PI3K-AKT Pathway as Combinatorial Target

Although the activating BRAF mutation is the most frequent somatic event in melanoma, and a valuable therapeutic target, several lines of evidence support that other pathways likely play a critical role in this disease. For example, benign nevi have a rate of BRAF V600 mutations that is similar to or higher than the rate observed in melanomas [86]. As benign nevi have an extremely low rate of malignant transformation, this finding demonstrates that other events must complement the BRAF mutation to fully explain the aggressive biology of this cancer. Of note, mutations in NRAS are also common in benign nevi [87]. In addition to this observation, functional studies in zebrafish, mice, and human cells have demonstrated that introducing expression of V600-mutant BRAF proteins alone in normal melanocytes fails to induce malignant transformation [88–90]. These model systems have provided a way to functionally interrogate candidates that may contribute to melanomagenesis. Finally, while many clinical specimens and cell lines with acquired resistance to selective BRAF inhibitor exhibit re-activation of the MAPK pathway, this has not

been a universal finding [69, 77, 91]. While many pathways remain to be interrogated, a number of studies support that the PI3K-AKT pathway can play an important role in this disease.

The PI3K-AKT pathway is a critical regulator of many cellular processes, including growth, survival, anchorage independence, motility/invasion, angiogenesis and metabolism, among others. The significance of the PI3K-AKT pathway in cancer is supported by the finding of a high rate of somatic alterations, including mutations, amplifications, and deletions, in multiple components of this pathway in many tumor types [92–94]. As described previously, activation of the PI3K-AKT pathway was initially implicated in melanoma by the identification of activating NRAS mutations and loss of function of the PTEN tumor suppressor. Interestingly, similar to previous comparison of PTEN loss and PI3K mutations, quantitative analysis demonstrated that PTEN loss correlates with significantly greater activation of AKT than NRAS mutations, as measured by expression of phosphorylated (activated) AKT protein, in melanoma cell lines and clinical specimens [95, 96]. While mutations in the catalytic subunit of PI3K, *PIK3CA,* are common in several tumor types, they are detected in only 1–2 % of melanomas [97]. Point mutations in the regulatory pleckstrin homology (PH) domain of AKT1 have been identified as rare events in several tumor types, including melanoma (~1 %) [98]. In addition, the analagous mutation in *AKT3* has been identified uniquely in melanoma [28]. This finding builds upon several other studies specifically implicating AKT3 as an important AKT isoform in this disease, whereas most research in other cancers implicates AKT1 and/or AKT2 [99–101]. Finally, mutations and amplifications of oncogenic receptor tyrosine kinases (RTKs) that activate signaling through the PI3K-AKT pathway in other cancers, such as HER2/neu and the epidermal growth factor receptor (EGFR), have not been detected as significant events in cutaneous melanomas, although aberrations of the KIT RTK have been implicated in other subtypes [32]. One report indicated that mutations throughout the sequence of the ERBB4 (HER4) gene were detected in ~20 % of melanomas [102]. Although this pattern of mutations was curious for a proposed oncogene, functional studies in cell lines with enforced expression of several of the variants detected in patients did suggest that the mutations were activating. However, recent whole exome sequencing efforts have not identified somatic mutations of ERBB4 as a significant event [42, 43].

A role for activation of the PI3K-AKT pathway in the transformation of melanocytes has been suggested primarily in preclinical models. In a genetically engineered mouse model (GEMM) in which inducible loss of PTEN in melanocytes was achieved with topical treatment with 4-hydroxytamoxifen, no melanocytic lesions were observed. In the same model, induction of the BRAF V600E mutation in newborn mice resulted in melanocyte hyperplasia, but no invasive lesions (melanomas) were observed. However, crossing of the two strains of mice to generate targeted expression of the BRAF V600E mutation with loss of PTEN expression in melanocytes resulted in invasive melanomas in all mice within 7–10 days of 4-hydroxtamoxifen treatment. In addition to being 100 % penetrant, the tumors formed spontaneous metastases in all of the mice. All mice required euthana-

sia within 25–50 days of induction [88]. Expression of an activated form of AKT3 (myr-AKT3) also transforms human melanocytes that express the BRAF V600E protein [100]. Interestingly, although NRAS mutations and genetic loss of PTEN are mutually exclusive in patients, loss of PTEN increased the metastatic potential and invasive behavior of NRAS-mutant melanomas in another GEMM [103].

Studies in advanced melanomas also support that PTEN loss is important functionally. In particular, a number of studies have compared BRAF-mutant human melanoma cell lines that lack PTEN to those that have normal PTEN function. Loss of PTEN correlates with increased activation of AKT in BRAF-mutant cell lines and tumors, and is also observed after knockdown of PTEN expression with RNAi [104]. Treatment of BRAF-mutant, PTEN-null human melanoma cell lines with BRAF or MEK inhibitors generally results in cytostatic effects, although one study identify a subset of resistant lines that also had loss of *Rb* [105]. In contrast to other BRAF-mutant cell lines, most of the cell lines with loss of PTEN fail to undergo apoptosis following treatment with BRAF or MEK inhibitors [104–107]. Resistance to apoptosis can also be induced in BRAF-mutant cell lines by inhibiting PTEN expression with RNAi [104]. These findings support that BRAF-mutant melanomas with loss of PTEN may exhibit at least some degree of de novo resistance to MAPK pathway inhibitors. Sequencing and copy number analysis of 34 patients enrolled in the phase I and phase II studies of dabrafenib detected aberrations in the PTEN gene in 11 (32%) of the patients [108]. Patients with PTEN loss had a similar rate of clinical response (36%) as those with genetically intact PTEN (43%). However, PTEN loss showed a very strong trend, even in this relatively small set of patients, for shorter PFS (18 weeks versus 32 weeks, $p=0.06$). Overall, analysis of samples collected at the time of disease progression found that homozygous deletion of PTEN was observed more frequently (4/10) than in the pre-treatment samples (2/34, $p=0.017$). A previous analysis of 5 patient that had matching pre-treatment and disease progression samples found discordance in 1 sample, which exhibited homozygous loss of PTEN at disease progression [74].

In addition to constitutive activation in melanomas with PTEN loss, it appears that activation of the PI3K-AKT pathway through growth factor receptors can mediate resistance to BRAF and MEK inhibitors. Characterization of two BRAF-mutant, PTEN-expressing human melanoma cell lines with de novo cell resistance to apoptosis induction demonstrated that these cell lines had similar degree and duration of inhibition of the MAPK pathway as cell lines destined to undergo apoptosis, but they were unique in that they developed marked activation of AKT after MEK inhibitor treatment [104]. Similar results were also observed subsequently with selective BRAF inhibitors [107]. Inhibition of the insulin-like growth factor 1 receptor (IGF1R), which both of the resistant cell lines expressed at high levels, abrogated the compensatory activation of AKT. Inhibition of IGF1R alone did not induce apoptosis in the cells, but marked cell death was observed when that was combined with MEK inhibition. This synergistic effect on apoptosis induction was recapitulated by knocking down AKT, or by inhibiting AKT activation with a dual TORC1/2 inhibitor, demonstrating that PI3K-AKT activation was mediating IGF1R-induced resistance.

Overexpression of IGF1R was also observed independently by investigators characterizing cell lines selected in vitro for secondary resistance to selective BRAF inhibitors [74]. These cell lines also demonstrated resistance to MEK inhibition by BRAF inhibitors through utilization of multiple RAF isoforms. While the MAPK pathway activation could be blocked in these cells by treatment with a MEK inhibitor, this failed to induce apoptosis in the resistant clones. Apoptosis was only seen with the MEK inhibitor when it was combined with a small molecule inhibitor of either IGF1R or PI3K. Analysis of matching samples from 5 patients treated with a selective BRAF inhibitor detected increased IGF1R expression in 2 patients at the time of disease progression (a third tumor had loss of PTEN). Resistant cell lines developed and characterized by another group of investigators also identified multiple RTKs that were upregulated at the time of resistance [69]. Although multiple RTKs were overexpressed (i.e. KIT, MET, EGFR), only the PDGFRβ was found to be activated by antibody array analysis. Increased activation of PDGFRβ was also identified in 4 (36%) of 11 patients with matching pre-treatment and progression samples following BRAF inhibitors. Functional testing demonstrated that the cell lines did not undergo apoptosis with MEK inhibitors alone, but did when MEK inhibitors were combined with either AKT or dual PI3K-mTOR inhibitors [109]. It is interesting to note that the two groups of investigators found completely non-overlapping RTKs mediating resistance in their different experimental systems. In addition, investigations by both groups failed to identify any mutations or amplifications of the genes encoding the implicated receptors [69, 74]. Thus, the induction of the RTKs appears to reflect an epigenetically-mediated mechanism of resistance.

While these studies identified resistance mechanisms that are intrinsic to the tumor cells, there is also evidence that activation of the PI3K-AKT pathway may be mediated in part by extrinsic factors. Two groups independently demonstrated that production of the growth factor HGF by stromal cells was capable of mediating resistance to BRAF inhibitors in BRAF-mutant human melanoma cells in co-culture systems [110, 111]. Supporting the clinical relevance of this finding, analysis of pre-treatment samples of patients treated with BRAF inhibitors demonstrated that increased expression of HGF in stromal cells correlated with a decreased chance of achieving a clinical response [110]. While not evaluated in patients, analysis of BRAF-mutant human melanoma cell lines showed that HGF did not rescue the cells from inhibition of MAPK signaling by BRAF inhibitors, but it induced PI3K-AKT pathway activation. The resistance mediated by exogenous HGF could be overcome by treating the cells with inhibitors of c-MET, the receptor for HGF, or with PI3K inhibitors.

The data implicating PTEN loss, RTK overexpression, and stromal growth factors together provide a strong rationale targeting the PI3K-AKT pathway in BRAF-mutant melanomas. Of note, data from these preclinical models suggests that only inhibiting the PI3K-AKT pathway is unlikely to be effective, due to both constitutive and compensatory activation of MAPK pathway signaling. In contrast, multiple studies have demonstrated that inhibition of the PI3K-AKT pathway can specifically sensitize cells to apoptosis induction by BRAF or MEK inhibitors [104–107,

109, 112]. In addition to increasing the degree of apoptosis, it appears that the timing of apoptosis induction is also shorter than what is observed with MAPK pathway inhibition alone. This suggests that intermittent dosing of PI3K-AKT pathway inhibitors may be an effective therapeutic strategy, which is supported by xenograft studies [113]. Examination of various dosing schedules may be critical to clinical development in this area, as the important role of the PI3K-AKT pathway in many basic physiological processes will likely make achieving an acceptable therapeutic index challenging. In contrast to the opportunity to target a tumor-specific activating mutation afforded by the BRAF V600 mutations in the MAPK pathway, activating mutations in the PI3K-AKT pathway are rare in melanoma. One possible route to improved therapeutic indices may be the use of isoform-specific inhibitors. For example, data in melanoma supports that the AKT3 isoform may be selectively important in melanoma progression, whereas its expression and function in most normal tissues appears to be rather limited [99, 114]. While inactivating mutations in PTEN are not directly targetable, two different studies have shown that PTEN loss may result in selective dependence on the β-isoform of the catalytic unit of PI3K (P110β, or *PIK3CB*) [115, 116]. As P110β appears to have a much more limited role in normal physiology, this may again allow for selective targeting of PTEN-null tumor cells, and thus an acceptable therapeutic index.

The clinical development of combinatorial strategies against the PI3K-AKT pathway is also complicated by several other factors [117, 118]. First, there are multiple different classes of inhibitors available to target the pathway, and generally multiple agents in each class undergoing clinical evaluation (Table 6.2). These classes include PI3K inhibitors (pan-PI3K and isoform-specific), dual PI3K-mTOR inhibitors, AKT inhibitors, TORC1 inhibitors (rapamycin-like agents), and dual TORC1/2 inhibitors. Previous studies support that different mechanisms of PI3K-AKT pathway activation can result in functional dependence on different effectors [96]. Thus, optimal clinical testing of the pathway may need to match the choice of therapeutic agent to the mechanism of pathway activation that is present in the patient. As the development of vemurafenib demonstrated, the rational testing and assessment of PI3K-AKT pathway inhibitors for melanoma would be facilitated by the identification of a reliable biomarker that correlates with efficacy/clinical benefit. However, while pharmacodynamic markers that do exist to determine if targets in the pathway have been inhibited, it still is unclear which targets, and what degree of target inhibition, are required for efficacy/synergy. Finally, studies in both patients and cell lines have demonstrated that the PI3K-AKT pathway is regulated by multiple feedback loops [119]. As a result, inhibition of a single target in the pathway may rapidly lead to a compensatory signaling mechanism that reactivates itself and/or other pathway effectors. Such feedback compensatory mechanisms have been observed with AKT, TORC1, and dual TORC1/2 inhibitors [120–122]. Thus, meaningful analysis of the effects of PI3K-AKT pathway inhibitors will likely require broad analysis of pathway markers in additional to pharmacodynamic evaluation of on-target effects.

Table 6.2 Classes of PI3K-AKT pathway inhibitors. GSK = GlaxoSmithKline. (Adapted with permission from [118])

Category	Examples
PI3K Inhibitors	BAY 80-6946 (Bayer) BKM120 (Novartis) GDC-0941 (Genentech) PX-866 (Oncothyreon) XL-147 (Exelixis) ZSTK474 (Zenyaku Kogyo)
PI3K: isoform-specific inhibitors	p110α-specific: BYL719 (Novartis) INK1117 (Intellikine) P110β-specific: GSK2636771 (GSK) SAR260301 (Sanofi) p110δ-specific: AMG 319 (Amgen) CAL-101 (Calistoga)
Dual PI3K/mTOR inhibitors	BEZ235, BGT226 (Novartis) GDC-0980 (Genentech) GSK2126458 (GSK) PF-4691502, PF-5212384 (Pfizer) SF-1126 (Semafore) XL765 (Exelixis)
AKT inhibitors	GDC-0068 (Genentech) GSK2110183 (GSK) MK-2206 (Merck) Perifosine (Keryx)
mTORC1 inhibitors	Everolimus (Novartis) Sirolimus (Pfizer) Ridaforolimus (Merck) Temsirolimus (Pfizer)
Dual mTORC1/2 inhibitors	AZD8055 (AstraZeneca) OSI-027 (Astellas)

6.6 Other Targets and Oncogenes

The clinical development of combinatorial approaches utilizing selective inhibitors of the V600-mutant BRAF protein is progressing rapidly as described. As these inhibitors may increase the growth of melanomas with a wild-type BRAF gene, these approaches are not likely to be applicable to patients without activating *BRAF* mutations. The non-V600 BRAF mutant population includes more than half of cutaneous melanoma patients, and even higher percentages of patients with other types of melanoma (i.e. acral, mucosal, and uveal). Thus, combinatorial strategies are also being developed for other targets that have been identified in this disease.

Activating mutations of *NRAS* are the second most common oncogenic somatic mutation detected in cutaneous melanomas. In addition to their prevalence, studies in both early- and late-stage melanoma patients support that melanoma patients with *NRAS* mutations have a worse prognosis than patients with activating *BRAF* mutations or wild-type *BRAF* and *NRAS* [17, 48]. Thus, the development of effec-

tive therapies for this subset of patients is a high priority. Direct targeting of RAS proteins is difficult to achieve due to the high affinity of the mutant RAS for GTP. Targeting RAS activation by inhibiting post-translational modifications that are required for its activation has been attempted in multiple tumor types, but to date this strategy has failed to produce clinical benefit [123]. As targeting RAS itself is challenging, multiple strategies have been developed to inhibit the multiple effector pathways that mediate its oncogenic effects [124, 125]. As activation of the RAS-RAF-MEK-ERK signaling cascade appears to be central to its effects, MEK inhibitors have been explored extensively as single agents and in combinations. A clinical trial with the MEK inhibitor binimetinib (MEK162) reported that clinical responses were observed in 28% of patients with activating *NRAS* mutations, while an additional 46% achieved disease stabilization [126]. However, the duration of disease control was quite short, and the overall median PFS was only 3.65 months. Multiple preclinical studies support that combined inhibition of MEK with targets in the PI3K-AKT pathway may be an effective strategy in RAS-mutant cancers, including melanoma [127–129]. Multiple clinical trials are currently ongoing testing this strategy. Recently, a GEMM of doxycycline-inducible mutant NRAS-expressing melanoma was used to compare the effects of MEK inhibitor treatment to complete extinction of NRAS signaling (doxycycline withdrawal) [130]. Surprisingly, the experiments demonstrated that MEK inhibition had similar efficacy to NRAS withdrawal in terms of apoptosis induction, but it was inferior at blocking cellular proliferation. Pathway analysis identified the cell cycle regulator CDK4 as a targetable node that correlated with this difference, and combined treatment with small molecule CDK4 inhibitors induced complete tumor regression in both the GEMM and in xenografts of NRAS-mutant human melanoma cells. Clinical trials will test the safety and efficacy of this strategy in patients. CDK4 is also an attractive combinatorial target in melanomas with activating BRAF mutations, as these tumors can have loss of P16, as well as activation of CDK4 (mutation or amplification) [42, 43]. Both loss of P16 and increased gene copy number of cyclin D1, another cell cycle regulator, correlated with shorter PFS in patients treated with dabrafenib in phase I/II clinical trials, providing further support for the clinical testing of this approach [108].

Activating mutations in *GNαQ* or *GNα11* are present in the majority of uveal melanomas, particularly those that have metastasized [36]. The most common mutations in these genes occur at the residue that is analogous to the Q61 residue of RAS proteins. Thus, similar to RAS, therapeutic development is generally focusing on effector pathways that are downstream of these mutations [35]. The initial characterization of *GNαQ* mutations demonstrated that this event activates signaling through the RAS-RAF-MEK-ERK signaling pathway. Preliminary results suggest that MEK inhibitors may be clinically effective in these patients. However, in vitro studies demonstrated that the efficacy of MEK inhibition may be compromised by compensatory activation of the PI3K-AKT pathway [131]. Combined treatment with MEK and PI3K inhibitors induced synergistic growth inhibition and apoptosis, supporting the rationale for testing of this combination in uveal melanoma. Testing is also ongoing with other effectors, including inhibitors of protein kinase C (PKC).

Alternatively, strategies to target growth factors and/or their receptors that are critical to growth in the liver, which is the most common metastatic site for uveal melanoma, are being evaluated clinically and preclinically [132].

6.7 Summary and Future Directions

The development of targeted therapy strategies for metastatic melanoma is evolving rapidly due to the improving understanding of molecular biology, new insights into the key determinants of clinical efficacy of targeted therapies, and the availability of multiple new agents against targets of interest. Based on emerging clinical and preclinical data, testing is rapidly moving from evaluation of single agents to rational combinatorial approaches. While this discussion has focused on the development specifically of combinations of multiple targeted therapies, the clinical management of melanoma patients generally utilizes multiple different therapeutic modalities. Experimental data supports that targeted therapies may synergize with many of these modalities, including chemotherapy, immunotherapy, and radiation [133–137]. In turn, the use of targeted therapy in combination with surgery, either in the adjuvant or neoadjuvant setting, has a strong rationale for development to see if this can improve cure rates in patients with clinically localized or regional disease. Thus, while the initial development of targeted therapy for melanoma has been highlighted by both successes and disappointments, the potential and future for this therapeutic approach remains bright.

Conflicts of Interest M.A.D. has served on advisory boards for GlaxoSmithKline, Genentech, Sanofi-Aventis, and Novartis, and has received research funding from GlaxoSmithKline, Genentech, AstraZeneca, Merck, Myriad, Sanofi-Aventis, and Oncothyreon.

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