Chapter

B-Raf-Mutated Melanoma

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Abstract

Until fairly recently, treatment options for advanced melanoma have been relatively limited. Fortunately, the last decade has seen dramatic improvements in response rates and duration of overall survival after the introduction of checkpoint inhibitors and targeted therapies against mutations in the B-isoform of Raf (B-Raf) in metastatic or inoperable melanoma. This book chapter will discuss the role of wild type B-Raf in the cell, the changes induced by mutations in this protein, and current FDA approvals for targeted therapies against B-Raf, both as a monotherapy and in combination with MEK inhibitors. We will also summarize mechanisms of resistance against these targeted therapies as well as novel therapeutic regimens proposed to bypass resistance.

Keywords: melanoma, metastasis, B-Raf, MEK, targeted therapy, adjuvant

1. Introduction

Among all malignancies, melanoma is the fifth most common cancer in men and the sixth most common in women in the USA. With 91,270 new cases diagnosed in 2018 and 9320 fatalities, it has the fastest increase in incidence of any cancer worldwide [1–4]. Although the majority of cases are treated with excision, approximately 30% of patients will progress to metastatic disease [5]. On average, 60% of patients with local metastases will survive up to 5 years, while only 15% of patients with distant metastases will have similar survival rates [6]. Prior to 2011, the only approved treatment options for metastatic diseases were dacarbazine or high dose interleukin-2 (IL-2). These therapies were associated with response rates of 10–20% and rarely prolonged overall survival in the population of patients with metastatic melanoma [7–9]. Fortunately, the last decade has seen dramatic progress in melanoma treatment through the identification and targeting of mutations in the rapidly accelerated fibrosarcoma protein (Raf) that is an essential mediator of the mitogen-activated protein kinase (MAPK) pathway. First identified as an oncogene in 2002, Raf mutations have been found in melanoma, colorectal cancer, papillary thyroid carcinoma, non-small cell lung cancer, multiple myeloma, hairy cell leukemia, and specific subset of astrocytomas, to name a few malignancies [5]. Up to 50% of melanoma patients were found to carry a mutation in the B isoform of Raf (B-Raf), suggesting that targeted therapy was a promising strategy in the treatment of this disease [6, 10].

2. Role of B-Raf in the cell

Wild type B-Raf is a serine/threonine-specific protein kinase that acts as an important component of the MAPK pathway regulating cellular proliferation,

survival, and differentiation. The B-Raf protein is composed of three main conserved regions that act by maintaining a closed conformation to autoinhibit protein function and to activate downstream pathway targets. Conserved region 1 (CR1) binds to conserved region 3 (CR3) to autoinhibit B-Raf function until activated by Ras. It also contains a zinc finger motif that aids in B-Raf docking at the cell membrane after activation. During activation, Ras binds to the CR1 domain allowing release of the bound CR3 domain. Conserved region 2 (CR2) acts to connect CR1 and CR3 and contains serine and tyrosine residues that are constitutively phosphorylated after Ras binding to help keep the protein in an open, active conformation and allow ATP binding. CR3 contains the enzymatic kinase domain of B-Raf, binding ATP and substrate proteins to catalyze the transfer of a phosphate group from ATP to the substrate, activating downstream signaling proteins. Importantly, this region also contains the valine amino acid at position 600, an amino acid that is often mutated resulting in the constitutive activation of B-Raf [11, 12].

B-Raf acts as a signaling protein in the Ras-Raf-Mek-Erk cascade, one of the most important oncogenic pathways in cancer. In wild type cells, extracellular growth factors and cytokines bind to transmembrane receptors on the cell's surface such as epidermal growth factor receptor (EGFR) and insulin like growth factor-1 receptor (IGF-1R). Intracellular phosphorylated sites on these receptors attract guanine nucleotide exchange factors (GEFs) such as SOS that bind to Ras and activate it by exchanging GDP for GTP. Once activated, Ras promotes the homo- and heterodimerization and activation of Raf kinases such as A-Raf, B-Raf and C-Raf. In turn, Raf kinases activate the MAP kinase pathway by phosphorylating MEK1 and MEK2. MEK proteins activate ERK 1 and 2 and the MAPK signaling pathway phosphorylates hundreds of downstream proteins [10, 13–16]. Importantly, activation of this pathway also sends inhibitory feedback towards upstream signaling components, which turn off signaling. This ultimately results in downregulation of Ras by ERK-dependent feedback [6]. Although the MAPK pathway is the most important downstream target of B-Raf, the JNK cascade, p38-MAPK pathway, and ERK-5 pathway have also been shown to be activated by B-Raf signaling [10].

3. Mutations in B-Raf that drive melanoma and their clinical significance

About 40–60% of melanomas will contain mutations in B-Raf at the V600 site, driving melanogenesis through upregulation of the Ras-Raf-Mek-ERK MAPK pathway [17]. Abnormal activation of the Ras-Raf-Mek Erk MAPK pathway is detected in approximately 90% of melanomas including the other genetic subsets such as Ras mutant, NF1 loss, and TWT [17]. Interestingly, other common mutations in melanoma, such as N-Ras, c-Kit, and NF1, also act through the MAPK pathway. B-Raf mutation alone is not considered sufficient to induce melanoma formation, as it has also been identified in benign and dysplastic nevi and can induce senescence [10, 18]. The vast majority (74–86%) of B-Raf mutations are substitutions of glutamic acid for valine at the 600th amino acid (V600E). However, substitutions of lysine for valine at amino acid 600 (V600K) in B-Raf is seen in 10–20% of melanomas and another 8% have other substations at the same site (V600M, V600D, and V600R) [6]. Case reports comparing the clinical significance of these different mutations show similar disease presentation and response to treatment [6, 9]. V600K mutations are more common in older patients and those with chronic sun exposure [9]. These B-Raf mutations occur in CR3 of the B-Raf protein and result in constitutive activation of the MAPK signaling pathway by destabilization of the inhibitory interaction between CR1 and CR3 through the introduction of a negatively charged or bulky amino acid at this site [10]. Mutations have also been identified in exon 15 (the region of DNA

adjacent to V600), exon 11 and translocations involving the B-Raf gene in melanomas and melanoma cell lines. Despite the alternative locations of these mutations, some can also act to drive melanogenesis through activation of the MAPK pathway, but do not signal as a monomer like V600 mutant proteins and in some cases require Ras activation [9].

Clinically, B-Raf mutations are associated with patients that are younger at initial diagnosis (<50 years old), locations with largely intermittent sun exposure, earlier diagnosis of distant metastasis (56 versus 63 years old), increased incidence of brain metastasis, a higher number of nevi and lesions with a truncal location. B-Raf mutations are not induced by UV (sun) DNA damage. Most concerning, some studies suggest that these mutations have been associated with shortened median survival (5.7 versus 8.5 months). However, these studies are often not powered to examine survival [10, 19–21]. This may relate to their association with increased ulceration in the tumor, a prognostic factor that independently is associated with decreased survival [22]. Additionally, B-Raf mutations are more common in superficial spreading or nodular subtypes of melanoma [10].

4. Diagnosis and diagnostic testing

Clinical detection of B-Raf mutations is a powerful tool in the management of advanced melanoma, allowing clinicians to make decisions regarding treatment plans with targeted therapy versus alternatives such as immunotherapy. Molecular testing for B-Raf mutations is recommended by both the National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO) guidelines in patients requiring systemic therapy [23–25]. This now includes most patients with stage III melanoma based on recent adjuvant trials. The sensitivity and specificity of the screening tests chosen is critical, as B-Raf detection and targeting is the only biomarker that can predict a therapeutic response to B-Raf inhibitor treatment in melanoma [23, 26]. Additionally, inappropriate treatment of B-Raf negative tumors with B-Raf inhibitors may be associated with tumor progression through paradoxical activation of the MAPK pathway based on numerous preclinical trials [27].

Due to advances in DNA sequencing, this method is being used more and more frequently as the initial method of mutation analysis. However, if there is not sufficient tissue or if rapid identification is needed diagnostic testing can be performed using immunohistochemistry with a VE1 monoclonal antibody to detect the V600E mutations. This method provides high sensitivity and is inexpensive. Unfortunately, it only detects this specific V600E mutation and misses other possible targets for B-Raf inhibitor therapy. Interpretation of immunohistochemistry by pathologists can also be subjective, making this method difficult to standardize. An alternative initial screening test is Sanger sequencing of the tumor DNA, often considered to be the gold standard. The tumor DNA is copied with amino acids attached to stop codons creating many copies of varying length that can be compared to determine the ultimate genetic sequence. This method is used less frequently, as a high ratio of mutant to wild type DNA is necessary to detect the B-Raf mutation and it has low sensitivity. If the immunohistochemistry or Sanger sequencing testing is negative it is often confirmed with pyrosequencing or RT-qPCR. Pyrosequencing is a method where DNA is sequenced using light tagged amino acids, allowing sequencing while the complementary DNA strand is being synthesized. This method is associated with a very high sensitivity for mutation detection; it also allows for quantification of mutated alleles in the tumor cell. However, pyrosequencing has a lower specificity than Sanger sequencing. Alternative confirmatory testing includes RT-qPCR,

another highly sensitive method that is relatively rapid and inexpensive but relies on primer design and selection for mutation detection and may miss uncommon mutations. As Next Generation Sequencing (NGS) becomes less expensive and more readily available in the clinic, it is becoming a more common method of mutation detection, allowing high sensitivity and specificity as well as the detection of rare mutations [6, 28]. Finally, studies evaluating levels of circulating tumor DNA show promise in evaluating disease response and relapse [28].

The above testing modalities are all laboratory based and are used in diagnostic centers that have been certified by the Clinical Laboratory Improvement Amendments and have been reviewed by the US Centers for Medicare and Medicaid Services. However, there are two testing modalities that were developed in concert with the testing and approval of targeted therapies that are considered companion diagnostic tests. These have been reviewed by the FDA and approved for diagnostic testing prior to initiation of these specific drug therapies. B-Raf mutations are detected using two primary companion diagnostic tests, the cobas 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc) and the THxID-BRAF kit (BioMerieux, Inc). Both RT-qPCR based, these tests were developed with vemurafenib plus cobimetinib and dabrafenib plus trametinib, respectively. Despite their high sensitivity, laboratory-based tests such as Sanger sequencing can be used to confirm negative results from companion diagnostic tests [28].

5. B-Raf inhibitor monotherapy

Given the frequency and importance of B-Raf in the development and progression of melanoma, interest in the development of B-Raf inhibitors was a high priority to all in the melanoma world. Three kinase inhibitors, vemurafenib, dabrafenib and encorafenib, are currently approved in the treatment of B-Raf V600-mutated melanoma. While they have been FDA approved in the treatment of B-Raf V600E and V600K-mutated melanomas, case studies and small trials suggest that these agents are also active in V600R mutants [6, 9]. However, based on published case reports, V600E mutations have improved response rates and longer progression-free survival after dabrafenib or vemurafenib treatment than other mutations [27].

Vemurafenib (PLX4032) is a B-Raf inhibitor that acts by binding to the ATP binding site in B-Raf, inhibiting the active form of the serine-threonine kinase [29, 30]. The BRIM3 trial was a phase III trial by Chapman et al. comparing vemurafenib targeted therapy (960 mg twice daily) with dacarbazine chemotherapy in 675 patients with untreated metastatic melanoma containing the B-Raf V600E or V600K mutation. The 6-month overall survival (OS) was 84% in the vemurafenib-treated group (95% confidence interval (CI) 78–89) versus 64% in the dacarbazine-treated group (95% CI, 56–73). Interim analysis showed a 63% reduction in the risk of death ($p < 0.001$) and a 74% reduction in the risk of either death or disease progression ($p < 0.001$) compared to dacarbazine. Overall response rates, a secondary endpoint, were 48% for vemurafenib and 5% for dacarbazine [31]. In follow up of this same population, McArthur et al. showed a median OS of 13.6 months in the vemurafenib-treated group (95% CI 12–15.2) versus 9.7 months in the dacarbazine-treated group (95% CI 7.9–12.8, $p < 0.001$). Progression-free survival (PFS) also improved with a median PFS of 6.9 months in the vemurafenib-treated group (95% CI 6.1–7) versus 1.6 months in the dacarbazine-treated group (95% CI 1.6–2.1, $p < 0.001$). Overall response rate increased with time to 57% in the vemurafenib group versus 9% in the dacarbazine group. Complete responses were seen in 6% of the vemurafenib-treated group versus 1% of the dacarbazine-treated group [32]. Based on these results, vemurafenib was the first approved drug for the treatment of B-Raf V600E and V600K-mutated advanced melanoma.

Dabrafenib (GSK2118436), another approved targeted therapy for the treatment of B-Raf V600-mutated melanoma, acts as a competitive inhibitor for ATP binding on the B-Raf protein and decreases its activity. Break-3 was a phase III trial by Hauschild et al. in which dabrafenib treatment (150 mg twice daily) was compared to dacarbazine administration. A total of 250 patients with previously untreated B-Raf V600E-mutated melanoma were enrolled. Dabrafenib therapy resulted in a median PFS of 5.1 months while the dacarbazine treatment group had a median PFS of 2.7 months. The hazard ratio for progression was 0.3 (95% CI 0.18–0.51, $p < 0.0001$). The OS hazard ratio was 0.61 (95% CI 0.25–1.48), suggesting significantly improved survival with dabrafenib treatment. About 50% of patients treated with dabrafenib had an objective response (95% CI 42.4–57.1) versus 7% with dacarbazine therapy (95% CI 1.8–15.5). Complete response was seen in 3% of patients treated with dabrafenib versus 2% of those treated with dacarbazine. Median time to response was 6.3 weeks (95% CI 6.1–6.3) with a median duration of response of 5.5 months. Patients that progressed on dacarbazine were allowed to cross over to treatment with dabrafenib, at the end of the study 44% of patients had crossed to dabrafenib treatment [33]. Based on these results, dabrafenib was approved by the FDA for treatment of B-Raf V600E-mutated advanced melanoma.

There has not been a direct head-to-head trial comparing dabrafenib and vemurafenib monotherapy in advanced melanoma with a B-Raf V600 mutation. However, extrapolating from the above trials suggests that they have very comparable clinical activity. Despite this, there is evidence suggesting that patients experience different drug-related toxicities. Vemurafenib was associated with toxicity requiring dose reduction due to grade 2 side effects in 38% of patients, while 28% of dabrafenib-treated patients required a dose reduction for grade 2 or greater side effects [32, 33]. Common toxicities of both drugs include rash, secondary skin malignancies (squamous cell carcinoma and keratoacanthomas), fatigue, arthralgia, and nausea. Vemurafenib was associated with higher rates of hepatic transaminitis, photosensitivity, and cutaneous hyperproliferative lesions; while, dabrafenib was associated with higher rates of pyrexia and chills. Despite the higher association with vemurafenib treatment, secondary skin hyperproliferative disorders and malignancies are seen with all B-Raf inhibitors. Median time to development of a squamous cell carcinoma after B-Raf inhibitor initiation is approximately 8 weeks and is seen in 20% of patients [10, 32]. These cutaneous side effects are primarily mediated by loss of feedback inhibition on the MAPK pathway after B-Raf suppression. In wild type cells, these B-Raf inhibitors accelerate B-Raf and C-Raf dimerization to activate the MAPK pathway. However, in B-Raf-mutated cells, signaling through negative feedback inhibition results in downregulation of MAPK signaling. After the addition of B-Raf inhibitors this negative feedback is lost, resulting in upregulation of MAPK signaling through C-Raf and Ras. Uncontrolled Ras activity has been associated with skin tumor formation, particularly squamous cell carcinomas. These patients are also at increased risk of new primary B-Raf wild type melanomas through similar mechanisms of action [6, 10].

B-Raf inhibition induces an overall response in approximately 50–60% of melanomas with B-Raf mutations. Predictors of response include B-Raf V600E mutations, higher PTEN levels at baseline (patients with deleted or mutant PTEN showed shorter PFS with dabrafenib therapy), initially increased levels of phosphorylated ERK followed by downregulation of phosphorylated ERK after treatment initiation, absence of MEK1p124 mutation, absence of CDKN2a gene deletion or chromosomal gains of the CCND1 gene [10]. Unfortunately, median progression-free survival with B-Raf targeted therapy is only 7 months [27, 31, 33]. Clinical factors that may be associated with a shorter PFS include an ECOG performance status of greater than 2, an elevated LDH at treatment initiation and M1C disease [10].

Even after clinical evidence of progression during treatment with vemurafenib or dabrafenib, studies suggest that continued treatment with B-Raf inhibitors may prolong survival through impedance of disease growth while preventing a disease flare that can be seen with cessation of treatment [10, 34, 35].

6. Combination therapy with B-Raf and MEK 1/2 inhibitors

Compared to the treatment modalities that were available prior to the development of targeted therapies, treatment with B-Raf inhibitors resulted in exceptional response rates and increases in overall survival. Investigation into inhibition at another downstream protein of the Ras-Raf-MEK-ERK MAPK pathway with MEK 1/2 inhibitors in the METRIC trials resulted in an overall response rate of approximately 30% and improved PFS of the oral selective MEK inhibitor trametinib when given orally (dose 2 mg) compared to treatment with dacarbazine in B-Raf-mutated melanoma [9, 36, 37]. Toxicities seen in the trial attributed to both drugs included rash, hypertension, diarrhea, edema, cardiac dysfunction, serum creatinine elevation, and ocular toxicities [10]. However, extrapolation from these studies and those of B-Raf inhibitors suggested that B-Raf inhibition was a more efficacious targeted therapy than MEK inhibition alone [38]. Relapse rates and side effect profiles with B-Raf inhibitor monotherapy were much higher than expected and were thought to be associated with reactivation of the MAPK pathway. For this reason, basic scientists and clinical investigators began combining B-Raf inhibitors with MEK inhibitors to block at two levels of this signaling pathway, intending to block any paradoxical activation after B-Raf inhibition [6]. Trametinib, cobimetinib, and bimimetinib are MEK inhibitors currently approved to be used in combination with B-Raf inhibitors in the treatment of advanced melanoma. Case reports also suggest that MEK inhibitors may be an effective therapy choice in patients with alternative mechanisms of MAPK activation, such as mutations in codons adjacent to that containing V600 [9].

The COMBI-DT, an initial phase II trial by Flaherty et al. evaluating dabrafenib and trametinib treatment in 247 patients with untreated B-Raf V600E- or V600K-mutated melanoma, found that dual targeted therapy (150 mg dabrafenib twice daily and 1 or 2 mg trametinib daily) resulted in a median PFS of 9.4 months versus 5.8 months for dabrafenib monotherapy. Median overall survival was 27.4 months for the combination therapy versus 20.2 months for the monotherapy. The hazard ratio for progression or death was 0.39 (95% CI 0.25–0.62, $p < 0.001$). Overall response was 76% in the dual therapy group compared to 54% with dabrafenib monotherapy ($p < 0.03$). Cutaneous side effects were significantly decreased with the addition of the MEK inhibitor [36, 37]. Following these results, phase III trials were performed showing similar outcomes. The COMBI-D phase III trial by Long et al. evaluated 423 patients with mutated B-Raf V600E or V600K advanced melanoma, who were treated with the combination of dabrafenib (150 mg twice daily) plus trametinib (2 mg daily) or dabrafenib alone. Median overall survival was 25.1 months in the combination group (95% CI 19.2–not reached) versus 18.7 months in the dabrafenib treatment group (95% CI 15.2–23.7, $p = 0.017$). Overall survival was 74% at 1 year and 51% at 2 years in the combination therapy group versus 68 and 42% in the monotherapy group. Median PFS was 11 months in the combination therapy group (95% CI 8–13.9) versus 8.8 months (95% CI 5.9–9.3, $p = 0.0004$). Rates of grade 3–4 adverse events were similar between both (32 versus 31%), pyrexia was the most common side effect with dabrafenib and trametinib combination therapy, while hyperkeratosis was the most common side effect in the dabrafenib alone group [39]. The COMBI-V study was a phase III trial

by Robert et al. evaluating combination dabrafenib and trametinib therapy against vemurafenib monotherapy. A total of 704 patients with untreated mutant B-Raf V600E or V600K were randomized to receive dabrafenib (150 mg twice daily) with trametinib (2 mg once daily) versus vemurafenib (960 mg twice daily). Overall survival at 1 year was 72% in the combination therapy group (95% CI 67–77) versus 65% in the vemurafenib alone group (95% CI 59–70). Hazard ratio for death with combination therapy was 0.69 (95% CI 0.53–0.89, p = 0.005). Median PFS was 11.4 months in the combination therapy group and 7.3 months in the vemurafenib monotherapy group (HR 0.56, 95% CI 046–0.69, p = 0.001). Objective response rates were 64% in the combination therapy group and 51% in the monotherapy group (p < 0.001). Similar to the COMBI-D trial, rates of severe adverse events were comparable but rates of squamous cell carcinoma and other skin complications were significantly higher with vemurafenib monotherapy [40]. Based on these results, combination therapy with dabrafenib and trametinib was approved by the FDA for treatment of B-Raf-mutated V600E and -V600K advanced melanoma.

Another MEK inhibitor, cobimetinib, has also shown efficacy in combination with the B-Raf inhibitor vemurafenib in advanced melanoma. The coBRIM trial, a phase III trial by Larkin et al., studied 495 patients with untreated B-Raf-mutated V600E and -V600K advanced melanoma treated with vemurafenib (960 mg twice daily, continuously) and cobimetinib (60 mg daily for 21 days followed by 7 days off) versus vemurafenib alone. Median PFS was 9.9 months in the combination therapy group versus 6.2 months in the control group. The hazard ratio for death or progression was 0.51 (95% CI 0.39–0.68, p < 0.001). Overall response rates were 68% in the combination group versus 45% in the monotherapy treatment group (p < 0.001) with 10% of patients in the combination group achieving complete response (versus 4% in the vemurafenib alone group). Rates of adverse events trended toward higher occurrence in the combination therapy group; however, the difference was not significant (65% versus 59%) and rates of secondary skin cancers were lower in the combination therapy group [41]. A follow up study of the same patient population found a median PFS of 12.3 months (95% CI 9.5–13.4) in the combination therapy group versus 7.2 months in the vemurafenib group (95% CI 5.6-7.5, p < 0.0001). Median overall survival was 22.3 months for cobimetinib and vemurafenib treatment (95% CI 20.3–not estimable) versus 17.4 months for the vemurafenib group (95% CI 15-19.8, p = 0.005). Serious adverse events were seen in 37% of the combination treatment patients versus 28% of the monotherapy patients, the most significant of which were pyrexia and dehydration [42].

Second generation B-Raf inhibitors such as encorafenib were also tested in clinical trials in combination with MEK inhibitors. These drugs are associated with a 10× longer half-life than vemurafenib or dabrafenib [43]. Phase I/II trials have shown that combination therapy with encorafenib and the MEK inhibitor, binimetinib in B-Raf-mutated melanoma resulted in a median PFS of 11.3 months (95% CI 7.4–14.6) [44]. The COLUMBUS trial by Dummer et al. evaluated 577 patients with unresectable stage III or IV B-Raf V600E- or V600K-mutated melanoma that were treatment naïve or had progressed on prior immunotherapy treated with encorafenib (450 mg daily) plus binimetinib (45 mg twice daily) or with encorafenib (300 mg daily) or vemurafenib (960 mg twice daily) monotherapy. Median PFS was 14.9 months (95% CI 11–18.5) in the combination encorafenib and binimetinib group versus 9.6 months in the encorafenib group (95% CI 7.5–14.8) and 7.3 months in the vemurafenib group (95% CI 5.6–8.2) (95% CI 0.41–0.71, HR 0.54, p < 0.0001). Overall responses were detected in 63% of patients in the combination therapy group versus 51% of patients with the encorafenib group and 40% of patients in the vemurafenib group [45]. Overall survival was 33.6 months (95% CI 24.4–39.2) in the combination therapy group versus 23.5 months (95%

CI 19.6–33.6) in the encorafenib group and 16.9 months in the vemurafenib group (95% CI 14–24.5) (HR 0.62, 95% CI 0.47–0.79, p < 0.0001) [46]. Adverse events in the combination therapy group included increased γ-glutamyltransferase, creatinine phosphokinase and hypertension. Encorafenib monotherapy was associated with palmoplantar erythrodysaesthesia syndrome, myalgia and arthralgia; while vemurafenib monotherapy was associated with arthralgia. Interestingly, combination therapy with encorafenib and binimetinib allowed a higher maximum tolerated dose of encorafenib, suggesting as with the other combinations of B-Raf and MEK inhibitors dual blockade of the MAPK pathway abrogates side effects associated with B-Raf inhibition alone. Fewer adverse events ultimately resulted in treatment discontinuation in the combination therapy group [45, 46]. Although it is difficult to compare end points between clinical trials, median PFS for encorafenib and binimetinib in the COLUMBUS trial was longer (14.9 months) than for either dabrafenib-trametinib in the COMBI-D (11 months) and COMBI-V (11.4 months) trials or for vemurafenib-cobimetinib in the coBRIM trial (12.3 months) [39, 40, 42, 45, 46]. This difference may be due to the longer half-life of encorafenib or it may also be the result of B-Raf treatment in a population of patients that did not all have access to immunotherapy due to local approved indications and regulations. This may have resulted in a group of patients on the COLUMBUS trial that was dissimilar to those studied in the other B-Raf inhibitor trials [45].

Overall, the above studies suggest that dual therapy with B-Raf and MEK inhibitors provides a longer PFS and increased overall response rates compared to B-Raf inhibition alone [38, 42, 47–50]. Most importantly, combination therapy is also associated with a modified side effect profile, particularly in those caused by reactivation of Ras and the MAPK pathway such as cutaneous squamous cell carcinomas [27]. Although the data suggest that encorafenib-binimetinib treatment may result in a slightly longer PFS, there is little direct evidence available to help clinicians pick between B-Raf/MEK inhibitor therapies [45, 46]. Therefore, the potential side effect profile may be helpful in guiding the decision. Approximately 50% of patients treated with dabrafenib and trametinib develop pyrexia, while 47% of patients treated with vemurafenib and cobimetinib develop significant photosensitivity [36, 40–42, 47]. Encorafenib and binimetinib dual therapy resulted in elevated γ-glutamyltransferase, creatinine phosphokinase, and hypertension [45, 46]. All combinations are associated with similar rates of MEK inhibitor-related toxicities such as serous retinopathy and left ventricular dysfunction [45, 46]. Other potential differences that may aid in picking therapy include the need to refrigerate trametinib and to take dabrafenib and trametinib on an empty stomach.

Monotherapy with a MEK inhibitor in B-Raf wild type tumors has been of great interest. Binimetinib treatment in melanomas with N-Ras mutations resulted in a PFS of 2.8 months (versus 1.5 months with dacarbazine), however overall survival was not improved [51, 52]. In vivo studies have also seen clinical activity from MEK inhibitor treatment in combination with CDK4/6 inhibitors, MDM2 antagonists, and PI3K/AKT inhibitors in melanoma [9]. Unfortunately, monotherapy with a MEK inhibitor such as trametinib after failure or B-Raf treatment showed no response [6, 10].

7. Adjuvant therapy with B-Raf inhibition

The above studies evaluated combined targeted therapy in advanced melanoma, where the patients were either not surgical candidates or had metastatic disease. However, investigators have also evaluated whether adjuvant targeted therapy after surgical resection may result in increased progression-free or overall survival.

The COMBI-AD trial by Long et al. was a phase III trial, where 870 patients with resected stage IIIA, IIB and IIIC B-Raf V600E- or V600K-mutated melanoma were randomly assigned to placebo or treatment with dabrafenib (150 mg twice daily) and trametinib (2 mg daily). Estimated relapse free survival rates at 3 years were 58% in the treatment group versus 39% in the placebo group (HR for death 0.47, 95% CI 0.39–0.58, $p < 0.001$). Overall survival at 3 years was 86% in the treatment group versus 77% in the placebo group (HR for death 0.57, 95% CI 0.42–0.79, $p = 0.0006$). Combination treatment with dabrafenib and trametinib also resulted in increased metastasis-free survival and lower rates of relapse. Despite the 53% improvement in relapse free survival and 43% improvement in overall survival, these improvements must be weighed against the 26% discontinuation rate due to adverse events (most frequently pyrexia and fatigue) [53]. Hauschild et al. confirmed these results in an extended follow up, evaluating relapse free survival rates at 3 and 4 years for dabrafenib and trametinib co-therapy versus placebo. At 3 years, relapse free survival rates were 59% for combination therapy (95% CI 55–64%) versus 40% in the placebo arm (95% CI 35–45%). At 4 years, relapse free survival rates were 54% for combination therapy (95% CI 49–59%) versus 38% in the placebo arm (95% CI 34–44%) [54]. Single agent vemurafenib (960 mg twice daily) as adjuvant therapy was also studied after resection in patients with stage IIC, IIIA, IIIB, or IIIC melanoma. Treatment resulted in a substantial but not significant increase in disease-free survival [55]. These new data suggest that B-Raf and MEK inhibition not only play an important role in the treatment of metastatic melanoma, but they also may provide benefit to patients with stage III disease after surgical resection.

8. B-Raf targeted therapy in brain metastases

As discussed previously, B-Raf inhibitor therapy is an effective treatment option for patients with inoperable or metastatic melanoma. Unfortunately, melanoma has one of the highest cerebral tropisms of any malignancy. Approximately 20% of stage IV patients have brain metastases at time of diagnosis and up to 40–50% of patients with stage IV melanoma will ultimately develop intracranial disease [56]. This development contributes significantly to mortality in 20–54% of metastatic melanoma patients; and once brain metastases are diagnosed, median survival decreases to 4–5 months [56–58]. Therefore, in evaluating the efficacy of targeted and immunotherapies in advanced melanoma, it is important to evaluate whether these agents are active in the central nervous system. The BREAK-MB trial showed that dabrafenib (150 mg twice a day) had an acceptable safety profile and induced a response in the metastatic brain lesions of 39% of B-Raf V600E mutant advanced melanoma if no prior local therapy had been used and in 31% of patients with prior local therapy. Median progression-free survival was 16 weeks and median overall survival was 31 weeks [59]. The COMBI-MB trial by Davies et al. was a phase II trial of dabrafenib (150 mg twice a day) and trametinib (2 mg daily) in 125 patients with V600 mutant melanoma. About 58% of patients with asymptomatic brain metastases and no prior therapy showed a response (95% CI 46–69) with a median progression-free survival of 5.6 months (95% CI 5.3–7.4) and a median overall survival of 10.8 months (95% CI 9.7–19.6). About 56% of patients who had received prior therapy showed a response with a median PFS of 7.2 month (95% CI 1.7–6.5), while 59% of patients with symptomatic brain metastases showed a response with a median PFS of 5.5 months (95% CI 2.8–7.3). About 44% of patients with V600D/K/R mutations responded to dabrafenib and trametinib with a median PFS of 4.2 months (95% CI 1.7–6.5) [60]. Vemurafenib has been studied in a phase 2 trial with similar results [57, 58]. Interestingly, these

trials show that there is a decreased response in the brain lesions when compared to extracranial lesions after B-Raf inhibition and overall the duration of response is approximately 50% that of extracranial sites, which may be due to higher concentrations of drug at the extracranial tumor site [61, 62].

Unfortunately, investigators have also found that the brain is a frequent site of disease recurrence or metastases after B-Raf inhibition [58]. This is thought to be related to signaling changes in the metastatic cell. MAPK downregulation is associated with upregulation of the PI3K/AKT pathway. Increased signaling through this pathway is often found in brain metastases [62, 63]. Therefore, it is also important to continue to investigate optimal treatment for intracranial disease after treatment with B-Raf inhibitors.

9. Mechanisms of resistance

Initial response rates to B-Raf inhibitors in B-Raf-mutated melanoma ranged between 50 and 70%, suggesting that 30–50% of these tumors have a mechanism of primary resistance prior to therapy. Additionally, approximately 50% of patients treated with B-Raf targeted therapy develop resistance within 1 year and only 10% of patients will respond to combination B-Raf and MEK targeted therapy for at least 3 years [10]. On average, resistance to B-Raf inhibition occurs after 6–8 months of treatment, although this is prolonged with dual MEK inhibition [38]. Evaluation of tumor samples after the development of B-Raf inhibitor resistance showed 38% of the mechanisms of resistance were non-genomic in origin, while 56% were due to both genomic and non-genomic changes [64]. About 79% of these mechanisms are associated with MAPK signaling reactivation [38]. Adjusting treatment regimens to address B-Raf inhibitor resistance is made even more difficult by the finding that several resistance mechanisms often coexist within the same tumor or between different tumor sites in patients treated with B-Raf inhibitors [27, 38].

Although mechanisms of primary resistance have been defined, it is difficult to conclusively establish that there was no response to treatment. Almost all patients with B-Raf-mutated melanoma respond initially to B-Raf inhibition; however, the duration of response is so short that there is evidence of progression at the time of disease evaluation. Alterations in the MAPK pathway such as predominance of signaling through C-Raf or the PI3K pathway increases immunity to B-Raf inhibition. NF1 is a tumor suppression that acts to inhibit Ras, and loss of NF1 function leads to constitutive Ras activation and activation of the MAPK pathway irrelevant of B-Raf inhibition. Through similar signaling changes, alterations in the PI3k-AKT-mTOR pathway (such as loss of function in PTEN) lead to constitutive activation of AKT and cell survival. Alterations in the RB1 pathway through mutations in cyclin D1, CDK4, or CDK6 can also lead to cell cycle progression irrelevant of B-Raf signaling [6].

Mechanisms of secondary resistance that develop after treatment with B-Raf inhibitors predominantly occur through changes allowing MAPK signaling despite B-Raf inhibition. Signaling through the MAPK pathway can be restored through N-Ras or MEK1/2 activating mutations. Upregulation and activation of the receptor tyrosine kinases and the PI3K-AKT-mTOR pathway (through IGF1-R, PDGFRβ, MET, mTORC1/2, EGFR, and ERBB3) can also activate MAPK signaling regardless of B-Raf inhibition. These changes have been identified in cell lines and in biopsies from the tumors of B-Raf inhibitor-treated patients after progression. Feedback activation of EGFR following B-Raf inhibition causes resistance through deactivation of MIG6 and increased expression of SOX10, restoring downstream signaling. But the most important pathways effect the B-Raf V600 molecule themselves,

including alternative splicing of the B-Raf V600E protein resulting in loss of the RAS binding domain and decreased sensitivity to the inhibitor as well as amplification of the B-RAF V600 gene inducing an overabundance of ligand. Copy number amplification of the B-Raf mutation can result in drug saturation and lead to dimerization despite inhibitor exposure, allowing downstream activation. Upregulation of C-Raf can increase signaling through a similar mechanism. MAP3K8 encodes COT, a protein that phosphorylates MEK independently of Raf signaling. Mutations in MAP3K8 have been identified in resistant tumors. Shifts in cellular metabolism to favor oxidative metabolism through increased expression of PGC1alpha have also been associated with B-Raf inhibition [6, 10]. Increased signaling through the YAP pathway and escape from cell death through upregulation of Bcl-XL have been identified in resistant cells after treatment with B-Raf inhibitors [9]. Mutations in the PI3K-AKT pathway (either through positive regulation of the pathway or negative regulation of its inhibitors PIK3R2 or PHLPP1) can upregulate signaling through this pathway, allowing cell survival despite B-Raf inhibition [6, 10]. The tumor microenvironment can also upregulate MAPK signaling through increased MAPK signaling in melanoma-associated fibroblasts after B-Raf inhibitor exposure. These fibroblasts act to promote matrix formation and remodeling, creating a protective environment for the tumor cell [38, 65]. In a study of 132 melanoma samples collected after the development of B-Raf inhibitor resistance, 20% had a N-Ras/K-Ras mutation, 16% had developed a B-Raf splice variant, 13% showed B-Raf amplification, 7% had a MEK1/2 mutation, and 11% developed an alteration in a non-MAPK signaling pathway [66]. Combined treatment with B-Raf and MEK inhibitors has shown development of resistance through similar mechanisms [67]. In fact, resistance after treatment with combination therapy is more often mediated through MAPK signaling reactivation than after treatment with B-Raf inhibitor monotherapy (82 versus 50%) [66, 67].

Due to the relative high rate of primary and secondary resistance to B-Raf inhibitors, alternative dosing schedules are being studied to see if these slow the rate of treatment escape. Intermittent dosing schedules show some promise in increasing the average time to progression for B-Raf-mutated melanomas treated with B-Raf inhibition [10].

10. Future directions

In vivo data and studies involving patient tumor samples have found that soon after B-Raf inhibitor initiation, immune activation is enhanced in the tumor microenvironment through multiple mechanisms [6]. The microphthalmia-associated transcription factor (MITF) is activated by MAPK signaling to suppress the expression of melanocyte-lineage antigens. Blockade of this pathway with B-Raf inhibitors upregulates expression of these melanoma-specific antigens, increasing the immune system's ability to recognize and target tumor cells. By the time tumor progression is noted on B-Raf inhibitors, these markers are usually downregulated and suppressed. B-Raf inhibition is also associated with an increase in tumor infiltrating lymphocytes early after treatment initiation. Finally, B-Raf inhibition often results in decreases in the immunosuppressive cytokines interleukin (IL)-6 and IL-8. Associated with a better tumor response to B-Raf inhibition, these findings suggest that adding immunotherapy or employing immunotherapy somewhere in the treatment course may be beneficial [38, 68]. Mouse studies have also demonstrated that treatment with dabrafenib, trametinib, and an anti-PD1 immunotherapy resulted in improved outcomes compared to either therapy alone [38, 69]. Attempts at combining vemurafenib and ipilimumab have been terminated

due to poor tolerability including fulminant hepatitis [6]. However, clinical investigators have been evaluating responses to alternative combinations of B-Raf and MEK inhibitors with checkpoint inhibitors. Ribas et al. performed a phase I study combining dabrafenib, trametinib, and an anti-PD-L1 monoclonal antibody MEDI4736. Six patients with B-Raf-mutated advanced melanoma were treated with either MED14736 (3 or 10 mg/kg IV every 2 weeks), dabrafenib (150 mg twice daily) and trametinib (2 mg daily), or trametinib alone. Thrombocytopenia was the main dose limiting toxicity identified. About 100% of patients had a response to combination therapy [70]. Another phase I trial combining atezolizumab, an anti-PD-L1 antibody (800 mg every 2 weeks), cobimetinib (60 mg daily) and vemurafenib (960 mg twice a day for the first 21 days, then 720 mg daily) in 34 patients with B-Raf-mutated advanced melanoma found that this combination resulted in a manageable and reversible safety profile. Partial or complete responses were detected in 85.3% of patients [71, 72]. Combination therapy in a phase 1/2 trial with pembrolizumab (2 mg/kg every 3 weeks), dabrafenib (150 mg twice daily) and trametinib (2 mg daily) in 15 patients with advanced B-Raf mutant melanoma found manageable and reversible dose limiting toxicities and adverse events. About 60% of patients had a complete or partial response [73]. Finally, a phase I trial of pembrolizumab (2 mg/kg every 3 weeks), dabrafenib (150 mg twice a day), and trametinib (2 mg daily) was compared with dabrafenib/trametinib treatment in 60 patients with B-Raf-mutated metastatic melanoma found a median PFS in the triple therapy group of 16 months (95% CI 8.6–21.5) versus 10.3 months in the dual therapy group (95% CI 7–15.6, HR 0.66, p-0.04287). Rates of response were also more durable, 60% of patients on triple therapy had responses that lasted over 18 months compared to 26% dual therapy [74]. Many other clinical trials are ongoing evaluating the clinical benefit of treatment with combinations of B-Raf inhibitors and immunotherapy (NCT02130466, NCT02967692, NCT02908672, NCT02858921, NCT02224781, NCT01656642, NCT01673854, NCT01940809, NCT02631447, NCT03235245, and NCT02902042). Some tissue and mouse studies indicate that MEK inhibition impairs T cell proliferation and localization to the tumor tissue, suggesting combination therapies with MEK inhibitors and immunotherapy may not be synergistic. However, these findings have not been recapitulated in clinical trials (NCT01767454) [9].

Additionally, immunotherapy monotherapy trials have shown that treatment with nivolumab and pembrolizumab in B-Raf mutant and B-Raf inhibitor refractory disease is associated with promising results [9, 38]. A small study of 19 pts showed an improved overall survival with a transition of therapy from vemurafenib to ipilimumab within 4 months of starting [10].

In addition to combination therapies, clinicians and scientists have been evaluating alternative methods to avoid or overcome resistance mechanisms after B-Raf therapy. These include using Bcl inhibitors to prevent cell escape through the YAP pathway, autophagy inhibitors that act through Bcl-2m Bcl-XL and Bcl-w, mTor inhibitors, ERK inhibitors, additional MAPK inhibitors, or Jak inhibitors to bypass other mechanisms of survival [9, 10].

11. Conclusions

Compared to other currently approved therapies for advanced melanoma, B-Raf inhibitors are associated with a rapid onset of tumor regression, often within 1–2 weeks of treatment [9]. The speed of response is particularly beneficial in patients with a rapidly progressive or high burden of disease, as well as those with a poor performance status. The ECOG 6134 trial (NCT02224781) is accruing in an

attempt to answer whether initial treatment with checkpoint inhibitors or targeted therapy is more beneficial. This is a crucial study for which an answer will provide vital evidence for the sequencing of immunotherapy and B-Raf targeted therapy.

The introduction of B-Raf inhibitors has been an important component in the revolution of melanoma treatment that has occurred in the last decade. Vemurafenib, dabrafenib, and encorafenib in combination with MEK inhibitors such as cobimetinib, trametinib, and binimetinib, have resulted in unprecedented overall responses and increases in survival. Combination therapy has also improved patient outcomes and decreased the likelihood of significant side effects such as new cutaneous malignancies. Testing for B-Raf mutations and treatment with B-Raf inhibitors is now standard of care in oncology clinics throughout the world. However, there is still significant ongoing work in management of tumor resistance mechanisms and combination and/or sequential regimens are being studied with immuno-oncologic agents, as an example, to try to further boost the efficacy, while maintaining an acceptable safety and tolerability profile.

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