

# Vemurafenib: Background, Patterns of Resistance, and Strategies to Combat Resistance in Melanoma

Arjun Dupati\*<sup>†</sup>, Liza Gill<sup>†</sup>

College of Human Medicine, Michigan State University, East Lansing, MI, USA

\*Corresponding Author: Arjun Dupati; dupatiar@gmail.com

**Introduction:** Finding an effective treatment for metastatic melanoma has posed a series of challenges. Vemurafenib, a B-RAF tyrosine kinase inhibitor, has been one of the most successful medications to date in the treatment of metastatic melanoma. B-RAF is a serine/threonine kinase that is a part of the RAS-RAF-MEK-ERK signal transduction pathway, which plays a pivotal role in cellular proliferation, differentiation, and survival. Mutations in the B-RAF protein lead to a deregulated activation of MAPK and ERK. The focus of this review article is resulting resistance to vemurafenib and its clinical implications on the treatment of metastatic melanoma. This paper aims to highlight mechanisms of vemurafenib resistance that have been observed so far and offer potential clinical approaches to overcome resistance.

**Methods:** PubMed, Google Scholar, and EMBASE were searched using the following free text terms: 'vemurafenib', 'vemurafenib resistance', 'vemurafenib tyrosine-kinase inhibitor', 'vemurafenib metastatic melanoma', 'vemurafenib alternatives', and 'vemurafenib cancer'. The Cochrane database was searched for randomized controlled trials and systematic reviews using the same search terms above. Two independent reviewers analyzed the search results and corresponding articles.

**Discussion:** Research over the last decade, most notably in the past 2 years, has revealed a multitude of mechanisms of resistance to vemurafenib. Resistance to therapy with vemurafenib in metastatic melanoma could be explained by the presence of cancer stem cells.

**Conclusion:** In order to effectively circumvent resistance, it would behoove clinicians to approach metastatic melanoma with a cocktail of inhibitors as opposed to monotherapy.

**Keywords:** vemurafenib; molecular targeted therapy; melanoma drug resistance; metastatic melanoma; tyrosine kinase inhibitor; melanoma treatment.

## INTRODUCTION

Finding an effective treatment for metastatic melanoma has posed a series of challenges.<sup>1</sup> Patients have historically had very few treatment options from which to choose. In 2011 alone, malignant melanoma, the fifth most common cancer in the US, caused over 9,000 deaths in the United States and 40,000 deaths worldwide.<sup>1–3</sup> While metastatic melanoma is the most common cause of skin cancer–associated deaths, it is only a small portion of all melanomas.<sup>4</sup> It takes approximately 3 years for the first metastases to appear clinically from the time a primary melanoma is diagnosed and more commonly occurs in older individuals in the head and neck regions.<sup>3,5</sup> However, 10–15% of patients already have metastases at the time of diagnosis.<sup>1</sup> The most common sites of metastases are the lymph nodes and the lungs (79%).<sup>3</sup> The highest incidence of melanoma occurs in countries with fair-skinned populations, such as those in Northern Europe, the US, Australia, and New Zealand, suggesting

that ultraviolet light acts as a potent carcinogen in melanoma.<sup>6</sup>

Vemurafenib, a B-RAF tyrosine kinase inhibitor, has been one of the most successful medications to date in the treatment of metastatic melanoma.<sup>4,7</sup> Patients taking vemurafenib have an 84% survival rate at 6 months.<sup>4</sup> The period of progression-free survival in vemurafenib is approximately 5.3 months and median overall survival has been observed to be 16 months.<sup>8</sup> Roughly, 40–70% of melanomas are positive for a B-RAF mutation.<sup>2,9,10</sup> B-RAF is a serine/threonine kinase that is a part of the RAS-RAF-MEK-ERK signal transduction pathway, which plays a pivotal role in cellular growth, proliferation, differentiation, and survival.<sup>6–8</sup> Mutations in the B-RAF protein lead to a deregulated activation of MAPK and ERK. Other hypotheses surrounding the effect of B-RAF mutations include some of the remaining 'hallmarks of cancer' and 'emerging hallmarks', including resisting cell death, sustaining

<sup>†</sup>Arjun Dupati and Liza Gill contributed equally to the production of this manuscript.

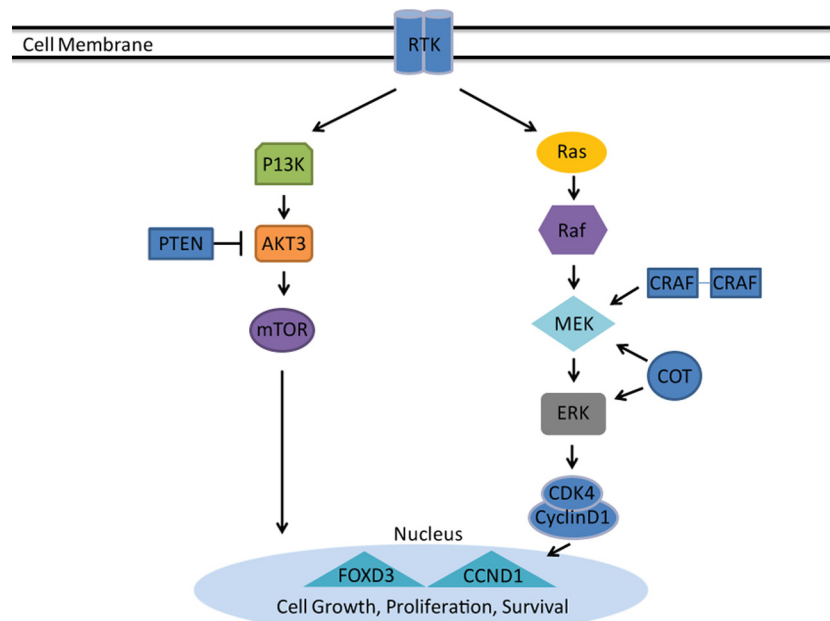
proliferative signaling, inducing angiogenesis, activating tissue invasion and metastasis, and evading immune destruction.<sup>3,8,11,12</sup>

Interestingly, B-RAF mutations are more commonly associated with melanomas occurring in regions of the body that are less frequently exposed to the sun, such as the trunk.<sup>1</sup> Mucosal and acral site melanomas rarely have B-RAF mutations.<sup>8</sup> Mutations in cyclinD1 are more commonly associated with melanomas occurring in areas with frequent sun exposure, such as the face and arms.<sup>1</sup> Mutations in the L597 and V600 (substitution of glutamic acid for valine at codon 600) locations of the *B-RAF* gene in exon 15 are most commonly associated with melanoma progression, warranting screening early in the disease process.<sup>1,2</sup> Since V600 mutations have been deemed as one of the most compelling reasons to use vemurafenib, some researchers have promoted the use of monoclonal antibodies to detect the particular mutation and ensure that all cases are observed.<sup>13</sup> V600E mutations have been more commonly associated with younger patients, whereas V600K mutations have been noted more often in older patients.<sup>1</sup> These mutations are present in the activating segment of the tyrosine kinase, offering a logical connection to cancer progression.<sup>2</sup>

Now, research shows that a combination therapy with B-RAF and MEK inhibitors shows greater promise than vemurafenib alone, likely conferred by increased mutation targeting.<sup>1</sup> The focus of this paper is resistance to vemurafenib and its clinical implications on the treatment of metastatic melanoma. An array of studies has shown that resulting resistance to vemurafenib is acquired by an intricate interaction between multiple cellular pathways.<sup>10</sup> Here we discuss some of those pathways (Fig. 1) and suggest potential clinical remedies to tackle the challenges of resistance.

## METHODS

PubMed, Google Scholar, and EMBASE were searched using the following free text terms: 'vemurafenib', 'vemurafenib resistance', 'vemurafenib tyrosine-kinase inhibitor', 'vemurafenib metastatic melanoma', 'vemurafenib alternatives', and 'vemurafenib cancer'. The Cochrane database was searched for randomized controlled trials and systematic reviews using these same search terms. Two independent reviewers analyzed the search results and corresponding articles. Many randomized control trials, review articles, and opinion pieces were included. Unpublished abstracts, conference proceedings, and current ongoing studies were excluded.



**Figure 1.** Simplified schematic of the MAPK and P13K pathways. When bound by their ligands, receptor tyrosine kinases activate RAS and P13K and their signaling cascades. The end result is survival, growth and proliferation of melanoma tumors. Mechanisms of BRAF-inhibitor resistance include, but are not limited to, PDGFR-beta upregulation, NRAS mutations, elevated CRAF, COT activation of ERK without the need for RAF signaling, loss of PTEN, CDK4 mutation and CCND1 amplification, CDK4 and cyclinD1 overexpression, AKT3 upregulation, and elevation of FOXD3. RTK: receptor tyrosine kinase (PDGFR-beta, IGF1-R, FGFR3).

The reference lists of included articles were analyzed to determine additional relevant articles. For those studies not accessible in full print, abstracts were obtained and analyzed. Only articles published in English were included. The reviewers were also limited to analyzing abstracts and articles available to them through their institution's journal subscription database. Discrepancies between reviewers were resolved by a collaborative review of the article in question and reaching a consensus.

## DISCUSSION

Despite the gains in survival made by vemurafenib in most B-RAF mutation-associated melanomas, researchers have observed that characteristics of resistance manifest as rapidly as the initial onset of the drug.<sup>6,14-17</sup> Resistance develops on average within 7 months of initial use.<sup>18</sup> Researchers have shown that resistance is generally not because of further adaptive mutations in B-RAF but rather mutations in genes coding for other important proteins.<sup>6</sup> This finding was confirmed with next-generation sequencing of 16 patients with clinical resistance to vemurafenib.<sup>6</sup> No secondary mutations were noted in the *B-RAF* gene. MAPK reactivation, noted in multiple studies of resistance, suggests other pathways (Fig. 1) play an integral role in the process of resulting resistance to vemurafenib.<sup>19</sup>

As mentioned before, resistance to vemurafenib generally occurs after an initial favorable response to the drug.<sup>20</sup> Research suggests that one form of resistance occurs as a result of upregulation of PDGFR-beta, a receptor tyrosine kinase (RTK), or NRAS mutations, a gene/protein associated with cell growth.<sup>20,21</sup> Specifically, the induction of a PDGFR-beta was shown to be a dominant feature of clinical resistance to vemurafenib.<sup>20</sup> Interestingly, those tumor cells found to have upregulated levels of PDGFR-beta exhibited low levels of RAS activity and demonstrated an insignificant increase in activity of MAPK with vemurafenib treatment. Tumor cells with high levels of NRAS, as a result of mutations, showed a significant increase in the activation of MAPK via hyperactivation of MEK-ERK1/2 pathway with vemurafenib treatment.<sup>20,22</sup> Such research helps support the hypothesis that, in order to treat vemurafenib-resistant melanomas, additional medications treating some of the other pathways discussed may be necessary to impede cancer growth. Studies attempting to determine the most effective treatment plan for melanoma proliferation, as a result of upregulation of PDGFR-beta, suggest the use of inhibitors of MEK1/2, PI3K, and mTOR1/2, which leads to the apoptosis of malignant

cells.<sup>23</sup> Just as B-RAF inhibitors lead to resistance, other studies show that MEK inhibitors, used alone, lead to MEK inhibitor resistance via B-RAF mutations and amplification.<sup>24,25</sup> This concept has been shown in both melanoma and colorectal cancer-associated B-RAF mutations.<sup>24,25</sup>

A suggested therapeutic strategy to avoid this form of resistance is to use B-RAF and MEK inhibitor simultaneously.<sup>24</sup> Trametinib, a MEK1/2 inhibitor, has recently received FDA approval for the treatment of BRAF-mutant metastatic melanoma.<sup>26</sup> A phase I/II trial combination of dabrafenib, a BRAF-inhibitor, and trametinib is already underway.<sup>26</sup> The therapeutic advantage of using a combination treatment plan is the prevention of cancer cells from acquiring other MEK or B-RAF mutations capable of circumventing directed monotherapy.<sup>27,28</sup> Concurrent treatment with a MEK-inhibitor and a BRAF-inhibitor also appears to result in less toxicity.<sup>26</sup> There are two phase 1 combinations of BRAF plus MEK inhibitors showing such reductions in severity of toxicity along with improvements in efficacy. However, it should be noted that a particular mutation, the MEK1 (C121S) mutation, which increases kinase activity, is resistant to both RAF and MEK inhibition in vitro.<sup>29</sup>

Monoclonal antibodies play a huge role in the treatment of a wide array of cancers and autoimmune diseases. CSPG4-specific monoclonal antibody, used with vemurafenib, has the ability to block multiple signaling pathways important to cell growth.<sup>30</sup> The addition of this particular monoclonal antibody has the added benefit of extending the amount of time vemurafenib has to exert its effects before resistance forms.<sup>30</sup>

Vemurafenib and ipilimumab, a monoclonal antibody directed against CTLA-4, were both approved by the FDA in 2011.<sup>26</sup> By binding CTLA4, ipilimumab enhances T-cell activation.<sup>26</sup> Evidence suggests that oncogenic B-RAF can be immunosuppressive, making the combination of a B-RAF-inhibitor with an immunotherapy a compelling proposition in the treatment of metastatic melanoma. In addition, treatment with MAPK inhibitors is associated with enhanced expression of melanocytic antigens, antigen recognition by T cells, and an influx of cytotoxic lymphocytes, creating more incentive to combine targeted and immune therapies. However, the phase 1 trial of vemurafenib plus ipilimumab had to be terminated due to toxicity concerns, particularly hepatic toxicity. Based on the earlier discussion of reduced toxicity using MEK and BRAF inhibitors, perhaps a triple combination with vemurafenib, ipilimumab, and trametinib would be a safer and more effective treatment.

Researchers have been able to successfully test hypotheses on the development of resistance in targeted cancer therapies using pre-clinical models with animals.<sup>31</sup> Such pre-clinical models have effectively predicted erlotinib resistance in EGFR-associated lung cancer, imatinib resistance in BCR-ABL leukemia, resistance to smoothed inhibitors in Patched1-deficient medulloblastoma, as well as ALK inhibitors in ALK-translocated lung cancers.<sup>31</sup> Some studies have shown tumor cells treated with vemurafenib to have high levels of ERK, even with low levels of MEK. Researchers have hypothesized that the high levels of ERK were a result of the activation of the PI3K/AKT pathway, thus increasing ERK levels via an alternative method. Subsequent inhibition of PI3K/AKT or ERK1/2 showed reduced cancer cell viability.<sup>32</sup> Those melanomas that appear to be refractory to both B-RAF and MEK inhibitors might benefit from a PI3K/AKT inhibitor or an ERK1/2 inhibitor.

Simultaneous mTOR activation has been noted in some studies with melanomas resistant to both MEK and B-RAF inhibitors. Consequently, the inclusion of an mTOR inhibitor along with a PI3K inhibitor to a treatment cocktail would be prudent. A dual PI3K-mTOR inhibitor has been shown to be superior to inhibition achieved by either mTOR inhibition or PI3K inhibition alone, perhaps by overcoming mTOR feedback loops.<sup>33,34</sup>

*MAP3K8*, the gene which encodes COT/Tpl2, is a MAPK pathway agonist that drives resistance to RAF inhibition in B-RAF (V600E) cell lines, thus conferring another viable way to evade long-term effective treatment using B-RAF inhibitors such as vemurafenib.<sup>9</sup> COT activates ERK without the need for RAF signaling. COT expression is associated with naturally inherent resistance in B-RAF (V600E) cell lines and acquired resistance in melanomas treated with both B-RAF and MEK inhibitors.<sup>9,21</sup> Perhaps, an addition of a COT inhibitor to a B-RAF and MEK inhibitor could prevent melanoma proliferation in cell lines identified as having a COT mutation.

Additional research has shown that some melanoma cell lines resistant to B-RAF inhibitors demonstrate elevated CRAF protein levels, which may play a significant role in resistance.<sup>35</sup> Researchers noted that a drug, known as geldanamycin, helps to degrade CRAF proteins, revealing its potential as an effective drug to overcome resistance in cases of refractory melanoma with elevated CRAF protein levels and resistance to vemurafenib.<sup>35</sup>

PTEN loss has been associated with resistance to B-RAF inhibitors in metastatic melanoma.<sup>36,37</sup> Up to

10% of melanomas resistant to a B-RAF inhibitor were shown to have a loss of PTEN expression.<sup>36</sup> PTEN does not play a role in cell growth, but rather plays a role in signaling for normal apoptosis of cells. Therefore, loss of PTEN expression predisposes cells to ineffective apoptotic signals.<sup>37</sup> PIX4720, a B-RAF inhibitor, was shown to stimulate AKT signaling in PTEN negative melanoma, but did not have the same effect in PTEN positive melanoma. Further investigation showed that the use of the B-RAF inhibitor increased BIM expression (a protein signal crucial for apoptosis) in PTEN positive melanoma, thus allowing for normal apoptotic signals to ensue, while PTEN negative melanomas did not have nearly as strong response. Furthermore, inhibition of BIM in PTEN positive melanoma revealed poor apoptosis in cell lines.<sup>36</sup> Studies have shown that apoptosis is dependent on the BH3-only proteins, Bim-EL and BMF, and inhibited by Mcl-1.<sup>38,39</sup> Treatment with XL888, an HSP90 inhibitor, increased BIM expression, decreased Mcl-1 expression, and successfully led to apoptosis in B-RAF inhibitor-resistant melanomas.<sup>40</sup>

Furthermore, AKT3 upregulation and activation has been associated with the survival of melanoma cells, especially in mutant B-RAF melanoma cells. Melanoma cell lines that were known to express higher levels of AKT3 were resistant to B-RAF inhibitor treatments.<sup>38</sup> Only after targeting AKT3 did B-RAF inhibitors, such as vemurafenib, effectively and successfully target the melanoma cell lines.<sup>38</sup> This reveals yet another mechanism by which B-RAF-mutant melanomas confer resistance to B-RAF inhibitors.

Recent studies have shown that there are considerable variations in response to treatment with B-RAF inhibitors. A study addressing the role of CDK4 and cyclin D1 in B-RAF inhibitor resistance in V600E cell lines showed that CDK4 mutations alone did not alter sensitivity.<sup>41</sup> However, cell lines with both a CDK4 mutation and *CCND1* amplification conferred B-RAF inhibitor resistance.<sup>41</sup> Researchers noted that as many as 17% of melanomas showed *CCND1* amplifications. Furthermore, cyclin D1 overexpression increased resistance, most notably when cyclin D1 and CDK4 were simultaneously overexpressed, revealing more therapeutic targets in the treatment of metastatic melanoma.<sup>41</sup> Recently, a number of selective CDK4/6 inhibitors have shown both tolerance and clinical benefit in clinical trials, opening the possibility of combinational therapies.<sup>42</sup>

Some B-RAF inhibitor resistant melanomas show increased IGF-1R/PI3K signaling. In such situations, treatment with IGF-1R/PI3K and MEK inhibitors leads to the desired cell death of resistant melanoma.<sup>43</sup>

It is suggested that increased levels of IGF-1R in post-relapse cancer cells are reflective of a survival mechanism dependent on the IGF-1R/PI3K pathway.<sup>43</sup>

Upstream activation may be a crucial component of vemurafenib resistant melanoma. One study's findings suggest that resistance to B-RAF (V600E) could occur due to elevated RAS-GTP levels and increased levels of AKT phosphorylation. Researchers insist that reactivation of the RAS/RAF pathway by upstream signaling activation plays a critical role in resistance to vemurafenib.<sup>44</sup> *FOXD3* is upregulated after inhibition of B-RAF-MEK signaling in mutant B-RAF melanoma. Research suggests that *FOXD3* elevation confers resistance. This was observed when siRNA knockdown of *FOXD3* led to greater apoptosis of the melanoma cell lines.<sup>45</sup> Elevation of *FOXD3* appears to be an adaptive mechanism for some forms of melanoma being treated with standard B-RAF and MEK inhibitors.<sup>45</sup>

Studies assessing the microenvironment of malignant cells reveal that secretion of hepatocyte growth factor (HGF) results in the eventual activation of MAPK and PI3K-AKT pathways, leading to B-RAF inhibitor resistance and uncontrolled proliferation.<sup>46</sup> One study quantified HGF levels secreted by surrounding stroma and stated that it strongly correlated with RAF inhibitor resistance.<sup>46</sup> Therapeutic management in this scenario could potentially be a RAF inhibitor coupled with an inhibitory compound for HGF. Further understanding of a malignant cell's environment could reveal other factors that predispose cancers to unabated proliferation.<sup>46</sup>

Research has shown that MEK/ERK reactivation via Ras signaling serves as a resistance mechanism in some melanomas.<sup>18</sup> Microarray confirmation demonstrates elevated Ras and RTK in resistant melanomas. Importantly, increased activation of FGFR3 correlated to Ras and MAPK activation, thus leading to vemurafenib resistance.<sup>18</sup> Researchers noted that inhibition of FGFR3 re-established sensitivity in resistant melanoma cell lines, further supporting their hypothesis.<sup>18</sup>

Vemurafenib is a targeted cancer therapy. Speaking generally about cancer drugs, such specificity of action has the benefit of having relatively fewer off-target effects and less nonspecific toxicity.<sup>12</sup> Scientific literature suggests that most targeted cancer therapies exhibit acquired resistance, especially with continuous dosing.<sup>47</sup> This phenomenon could be explained by growing evidence that each 'hallmark of cancer' (sustaining proliferative signaling, evading growth suppression, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis) is regulated by partially redundant signaling

pathways.<sup>48</sup> Thus, inhibiting only one key pathway in a tumor might allow some cells to survive and function until they or their progeny eventually adapt to utilize a different signaling pathway due to selective pressure of the imposed therapy. This adaptation property of cancers suggests that successful targeted therapy can only be achieved after elucidating alternative pathways leading to proliferation of cancer cells despite treatment, so that medications can be developed to target the involved proteins.<sup>48</sup> Even after targeting alternative signaling pathways, it is possible that cancer cells may also reduce their dependence on a particular hallmark capability and become more dependent on another. This has been seen in antiangiogenic therapies where clinical responses have been transitory. In some pre-clinical models, potent angiogenesis inhibitors show initial success, but the tumors then shift from a dependence on angiogenesis to invasiveness and metastasis. Such a transition leads the cancer cells from hypoxic conditions to well oxygenated, preexisting tissue vasculature. This preclinical model has been validated in human glioblastomas treated with antiangiogenic therapies. This kind of adaptation ability of tumors needs to be considered when developing cancer therapies, including therapy for metastatic melanoma.

Resistance to therapy with vemurafenib in metastatic melanoma could be explained by the presence of cancer stem cells. Evidence suggests that a variety of tumors contain a subpopulation of cells called cancer stem cells. Cancer stem cells are defined as cells that are efficient in initiating tumors upon xenotransplantation.<sup>12</sup> Cancer stem cells have the ability to self-renew along with the capacity to generate progeny at various levels of differentiation.<sup>49</sup> It seems that cells with properties of cancer stem cells are more resistant to common chemotherapeutic agents.<sup>12</sup> In addition to chemotherapy resistance, presence of cancer stem cells might explain disease recurrence, sometimes years to decades following apparently successful debulking of solid human tumors by radiation or chemotherapy.<sup>12</sup> The presence of a stem cell subpopulation in melanomas has been demonstrated.<sup>50</sup> Identifying and targeting this population of cells, in addition to treatment with vemurafenib, might lead to more effective treatments for metastatic melanoma.

A major limitation in this review includes the inability to access all full articles since articles were excluded if they were not written in English or unavailable to the reviewers through their institution's journal subscription database. The number of articles unavailable

through the institution journal subscription database was not recorded.

## CONCLUSION

Vemurafenib shows an 84% survival rate at 6 months.<sup>4</sup> The period of progression free survival in vemurafenib is approximately 5.3 months and median overall survival for vemurafenib has been observed to be 16 months.<sup>8</sup> Resistance to vemurafenib develops on average within 7 months of initial use.<sup>18</sup>

A myriad of different pathways for resistance have been highlighted in this review of B-RAF inhibition of malignant melanoma, many of which have specific molecular inhibitors that can be utilized with human drug therapy. Combination therapy of B-RAF inhibitors and other targeted drugs may either prevent or modify the ability for the cancer to exhibit resistance, and potentially prolong life and decrease complications. We suggest that physicians consider utilizing this breadth of information to tailor therapies for patients with malignant melanoma, and if human trials are not currently underway, that clinicians and scientists work together to develop new treatment regimens. Clearly there are many different pathways for resistance highlighted in this review; one pathway may not be a suitable treatment for every patient, but a physician must determine the advantages and disadvantages of specific therapies with their patient as multiple drugs often carry higher risks and complications than single drug therapy.

**Conflict of interest and funding:** Both authors are affiliated with the Medical Student Research Journal.

**Sources of Support:** None

## REFERENCES

1. Lemech C, Infante J, Arkenau HT. The potential for BRAF V600 inhibitors in advanced cutaneous melanoma: rationale and latest evidence. *Ther Adv Med Oncol* 2012; 4(2): 61–73. <http://dx.doi.org/10.1177/1758834011432949>.
2. Dahlman KB, Xia J, Hutchinson K, et al. BRAF(L597) mutations in melanoma are associated with sensitivity to MEK inhibitors. *Cancer Discov* 2012; 2(9): 791–7.
3. Castellani E, Covarelli P, Boselli C, et al. Spontaneous splenic rupture in patient with metastatic melanoma treated with vemurafenib. *World J Surg Oncol* 2012; 10: 155. <http://dx.doi.org/10.1186/1477-7819-10-155>.
4. De Mello RA. Metastatic melanoma and vemurafenib: novel approaches. *Rare Tumors* 2012; 4(2): e31. <http://dx.doi.org/10.4081/rt.2012.e31>.
5. Kim J, Lazar AJ, Davies MA, et al. BRAF, NRAS and KIT sequencing analysis of spindle cell melanoma. *J Cutan Pathol* 2012; 39(9): 821–5. <http://dx.doi.org/10.1111/j.1600-0560.2012.01950.x>.
6. Fisher R, Larkin J. Vemurafenib: a new treatment for BRAF-V600 mutated advanced melanoma. *Cancer Manage Res* 2012; 4: 243–52. <http://dx.doi.org/10.2147/CMAR.S25284>.
7. Flaherty KT, Yasothan U, Kirkpatrick P. Vemurafenib. *Nat Rev Drug Discov* 2011; 10(11): 811–12. <http://dx.doi.org/10.1038/nrd3579>.
8. Ascierto PA, Kirkwood JM, Grob JJ, et al. The role of BRAF V600 mutation in melanoma. *J Transl Med* 2012; 10: 85. <http://dx.doi.org/10.1186/1479-5876-10-85>.
9. Johannessen CM, Boehm JS, Kim SY, et al. COT drives resistance to RAF inhibition through MAP kinase pathway reactivation. *Nature* 2010; 468(7326): 968–72. <http://dx.doi.org/10.1038/nature09627>.
10. Kudchadkar R, Paraiso KH, Smalley KS. Targeting mutant BRAF in melanoma: current status and future development of combination therapy strategies. *Cancer J* 2012; 18(2): 124–31. <http://dx.doi.org/10.1097/PPO.0b013e31824b436e>.
11. Fedorenko IV, Paraiso KH, Smalley KS. Acquired and intrinsic BRAF inhibitor resistance in BRAF V600E mutant melanoma. *Biochem Pharmacol* 2011; 82(3): 201–9. <http://dx.doi.org/10.1016/j.bcp.2011.05.015>.
12. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144(5): 646–74. <http://dx.doi.org/10.1016/j.cell.2011.02.013>.
13. Skorokhod A, Capper D, von Deimling A, Enk A, Helmbold P. Detection of BRAF V600E mutations in skin metastases of malignant melanoma by monoclonal antibody VE1. *J Am Acad Dermatol* 2012; 67(3): 488–91. <http://dx.doi.org/10.1016/j.jaad.2012.03.022>.
14. Poulidakos PI, Rosen N. Mutant BRAF melanomas – dependence and resistance. *Cancer Cell* 2011; 19(1): 11–15. <http://dx.doi.org/10.1016/j.ccr.2011.01.008>.
15. Wilson TR, Fridlyand J, Yan Y, et al. Widespread potential for growth-factor-driven resistance to anticancer kinase inhibitors. *Nature* 2012; 487(7408): 505–9. <http://dx.doi.org/10.1038/nature11249>.
16. Dummer R, Flaherty KT. Resistance patterns with tyrosine kinase inhibitors in melanoma: new insights. *Curr Opin Oncol* 2012; 24(2): 150–4. <http://dx.doi.org/10.1097/CCO.0b013e3182834fca92>.
17. Aplin AE, Kaplan FM, Shao Y. Mechanisms of resistance to RAF inhibitors in melanoma. *J Invest Dermatol* 2011; 131(9): 1817–20. <http://dx.doi.org/10.1038/jid.2011.147>.
18. Yadav V, Zhang X, Liu J, et al. Reactivation of mitogen-activated protein kinase (MAPK) pathway by FGF receptor 3 (FGFR3)/Ras mediates resistance to vemurafenib in human B-RAF V600E mutant melanoma. *J Biol Chem* 2012; 287(33): 28087–98. <http://dx.doi.org/10.1074/jbc.M112.377218>.
19. Flaherty KT. Throwing the kitchen sink at melanoma drug development. *Pigment Cell Melanoma Res* 2012; 25(5): 543–4.

20. Nazarian R, Shi H, Wang Q, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature* 2010; 468(7326): 973–977. <http://dx.doi.org/10.1038/nature09626>.
21. Gowrishankar K, Snoyman S, Pupo GM, Becker TM, Kefford RF, Rizos H. Acquired resistance to BRAF inhibition can confer cross-resistance to combined BRAF/MEK inhibition. *J Invest Dermatol* 2012; 132(7): 1850–9. <http://dx.doi.org/10.1038/jid.2012.63>.
22. Kaplan FM, Shao Y, Mayberry MM, Aplin AE. Hyperactivation of MEK-ERK1/2 signaling and resistance to apoptosis induced by the oncogenic B-RAF inhibitor, PLX4720, in mutant N-RAS melanoma cells. *Oncogene* 2011; 30(3): 366–71. <http://dx.doi.org/10.1038/onc.2010.408>.
23. Shi H, Kong X, Ribas A, Lo RS. Combinatorial treatments that overcome PDGFR $\beta$ -driven resistance of melanoma cells to V600EB-RAF inhibition. *Cancer Res* 2011; 71(15): 5067–74. <http://dx.doi.org/10.1158/0008-5472>.
24. Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA. BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. *Sci Signal* 2010; 3(149): ra84. <http://dx.doi.org/10.1126/scisignal.2001148>.
25. Little AS, Balmanno K, Sale MJ, et al. Amplification of the driving oncogene, KRAS or BRAF, underpins acquired resistance to MEK1/2 inhibitors in colorectal cancer cells. *Sci Signal* 2011; 4(166): ra17. <http://dx.doi.org/10.1126/scisignal.4170er2>.
26. Sullivan RJ, Lorusso PM, Flaherty KT. The intersection of immune-directed and molecularly targeted therapy in advanced melanoma: where we have been, are, and will be. *Clin Cancer Res* 2013; 19(19): 5283–91. <http://dx.doi.org/10.1158/1078-0432.CCR-13-2151>.
27. Emery CM, Vijayendran KG, Zipser MC, et al. MEK1 mutations confer resistance to MEK and B-RAF inhibition. *Proc Natl Acad Sci USA* 2009; 106(48): 20411–16. <http://dx.doi.org/10.1073/pnas.0905833106>.
28. Nissan MH, Solit DB. The “SWOT” of BRAF inhibition in melanoma: RAF inhibitors, MEK inhibitors or both? *Curr Oncol Rep* 2011; 13(6): 479–87. <http://dx.doi.org/10.1007/s11912-011-0198-4>.
29. Wagle N, Emery C, Berger MF, et al. Dissecting therapeutic resistance to RAF inhibition in melanoma by tumor genomic profiling. *J Clin Oncol* 2011; 29(22): 3085–96. <http://dx.doi.org/10.1200/JCO.2010.33.2312>.
30. Yu L, Favoino E, Wang Y, Ma Y, Deng X, Wang X. The CSPG4-specific monoclonal antibody enhances and prolongs the effects of the BRAF inhibitor in melanoma cells. *Immunol Res* 2011; 50(2–3): 294–302. <http://dx.doi.org/10.1007/s12026-011-8232-z>.
31. Corcoran RB, Settleman J, Engelman JA. Potential therapeutic strategies to overcome acquired resistance to BRAF or MEK inhibitors in BRAF mutant cancers. *Oncotarget* 2011; 2(4): 336–46.
32. Jiang CC, Lai F, Thorne RF, et al. MEK-independent survival of B-RAFV600E melanoma cells selected for resistance to apoptosis induced by the RAF inhibitor PLX4720. *Clin Cancer Res* 2011; 17(4): 721–30. <http://dx.doi.org/10.1158/1078-0432.CCR-10-2225>.
33. Atefi M, von Eeuw E, Attar N, et al. Reversing melanoma cross-resistance to BRAF and MEK inhibitors by co-targeting the AKT/mTOR pathway. *PLoS One* 2011; 6(12): e28973. <http://dx.doi.org/10.1371/journal.pone.0028973>.
34. Greger JG, Eastman SD, Zhang V, et al. Combinations of BRAF, MEK, and PI3K/mTOR inhibitors overcome acquired resistance to the BRAF inhibitor GSK2118436 dabrafenib, mediated by NRAS or MEK mutations. *Mol Cancer Ther* 2012; 11(4): 909–20. <http://dx.doi.org/10.1158/1535-7163.MCT-11-0989>.
35. Montagut C, Sharma SV, Shioda T, et al. Elevated CRAF as a potential mechanism of acquired resistance to BRAF inhibition in melanoma. *Cancer Res* 2008; 68(12): 4853–61. <http://dx.doi.org/10.1158/0008-5472.CAN-07-6787>.
36. Paraiso KH, Xiang Y, Rebecca VW, et al. PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. *Cancer Res* 2011; 71(7): 2750–60. <http://dx.doi.org/10.1158/0008-5472.CAN-10-2954>.
37. Koomen JM, Smalley KS. Using quantitative proteomic analysis to understand genotype specific intrinsic drug resistance in melanoma. *Oncotarget* 2011; 2(4): 329–35.
38. Shao Y, Aplin AE. Akt3-mediated resistance to apoptosis in B-RAF-targeted melanoma cells. *Cancer Res* 2010; 70(16): 6670–81. <http://dx.doi.org/10.1158/0008-5472.CAN-09-4471>.
39. Shao Y, Aplin AE. BH3-only protein silencing contributes to acquired resistance to PLX4720 in human melanoma. *Cell Death Differ* 2012; 19(12): 2029–39. <http://dx.doi.org/10.1038/cdd.2012.94>.
40. Paraiso KH, Haarberg HE, Wood E, et al. The HSP90 inhibitor XL888 overcomes BRAF inhibitor resistance mediated through diverse mechanisms. *Clin Cancer Res* 2012; 18(9): 2502–14. <http://dx.doi.org/10.1158/1078-0432.CCR-11-2612>.
41. Smalley KS, Lioni M, Dalla Palma M, et al. Increased cyclin D1 expression can mediate BRAF inhibitor resistance in BRAF V600E-mutated melanomas. *Mol Cancer Ther* 2008; 7(9): 2876–83. <http://dx.doi.org/10.1158/1535-7163.MCT-08-0431>.
42. Sheppard KE, McArthur GA. The cell-cycle regulator CDK4: an emerging therapeutic target in melanoma. *Clin Cancer Res* 2013; 19(19): 5320–8. <http://dx.doi.org/10.1158/1078-0432.CCR-13-0259>.
43. Villanueva J, Vultur A, Lee JT, et al. Acquired resistance to BRAF inhibitors mediated by a RAF kinase switch in melanoma can be overcome by cotargeting MEK and IGF-1R/PI3K. *Cancer Cell* 2010; 18(6): 683–95. <http://dx.doi.org/10.1016/j.ccr.2010.11.023>.
44. Su F, Bradley WD, Wang Q, et al. Resistance to selective BRAF inhibition can be mediated by modest upstream pathway activation. *Cancer Res* 2012; 72(4): 969–78. <http://dx.doi.org/10.1158/0008-5472.CAN-11-1875>.
45. Basile KJ, Abel EV, Aplin AE. Adaptive upregulation of FOXD3 and resistance to PLX4032/4720-induced cell death

in mutant B-RAF melanoma cells. *Oncogene* 2012; 31(19): 2471–9. <http://dx.doi.org/10.1038/onc.2011.424>.

**46.** Straussman R, Morikawa T, Shee K, et al. Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. *Nature* 2012; 487(7408): 500–4. <http://dx.doi.org/10.1038/nature11183>.

**47.** Sosman JA, Kim KB, Schuchter L, et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 2012; 366(8): 707–14. <http://dx.doi.org/10.1056/NEJMoa1112302>.

**48.** Villanueva J, Vultur A, Herlyn M. Resistance to BRAF inhibitors: unraveling mechanisms and future treatment options. *Cancer Res* 2011; 71(23): 7137–40. <http://dx.doi.org/10.1158/0008-5472.CAN-11-1243>.

**49.** Elyer CE, Rich JN. Survival of the fittest: cancer stem cells in therapeutic resistance and angiogenesis. *J Clin Oncol* 2008; 26(17): 2839–45. <http://dx.doi.org/10.1200/JCO.2007.15.1829>.

**50.** Fang D, Nguyen TK, Leishear K, et al. A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 2005; 65(20): 9328–37.