

## IN-DEPTH REVIEW

### Current Therapy for Advanced Melanoma and a Look at Future Signaling Pathways to Target

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#### ABSTRACT

Metastatic melanoma is a heterogeneous tumor of the skin derived from melanocytes notorious for its resistance to various forms of systemic therapy. Many different types of monotherapies and combination therapies have been developed in recent years, each with their own setbacks, and drawbacks. This review article will provide an overview of current FDA approved drug therapies for stage III and IV metastatic melanoma, key signaling pathways they target, and mechanisms of drug resistance. This paper will then look at future therapy for metastatic melanoma with a particular focus on targeted therapy on embryonic and evolutionarily conserved pathways in metastatic melanoma, including notch, wnt, hippo, hedgehog signaling, among others.

#### INTRODUCTION

Melanoma is a deadly skin cancer arising from melanocytes, the cells in the basal layer of the epidermis responsible for forming the pigment in our skin, hair and eyes. Melanoma's incidence has continuously increased throughout the years.<sup>1</sup> Due to public awareness and advancements in diagnostic methods, cases of melanoma frequently are diagnosed earlier through surgical excision, and thus carry a good prognosis and 5-year survival rate.<sup>2</sup> However, advanced metastatic stages of melanoma (III and IV) have proven to be difficult to treat, with traditional chemotherapeutic drugs like dacarbazine

(DTIC) proving ineffective. In more recent years, targeted therapy, most famously, vemurafenib, a drug that targets melanomas harboring a V600E mutation, have shown improvement and promise in treating metastatic melanoma.<sup>1</sup> Even still, metastatic melanoma has proven resilient to new treatments with its unique, heterogenous resistance mechanisms, requiring new approaches to bypass resistance.<sup>3</sup>

***Interferon alpha-2b***

Traditional therapy for melanoma includes surgery, radiation and systemic therapy, the latter a category which encompasses chemotherapy and immunotherapy.<sup>4</sup> Immunotherapy enhances the body's immune function to combat tumors. FDA approved management options for resected stage III melanoma immunotherapy includes interferon alpha-2b. Interferon alpha-2b has multiple actions in the body, including binding to cell receptors and subsequently initiating increased phagocytic activity of macrophages and increased cytotoxicity of lymphocytes. Interferon alpha-2b has shown benefit in relapse-free survival benefit (1.72 vs 0.98 years), but no significant difference in overall survival (OS).<sup>5</sup>

***Interleukin-2***

FDA approved immunotherapy options for stage IV melanoma includes interleukin-2 (IL-2). IL-2 is a T-cell growth factor. Treatment with IL-2 carries only a 16% overall response rate with a 6% complete response.<sup>6</sup>

***Anti-CTLA-4 Antibody***

More recent research has focused on activating adaptive and innate immune responses against tumor antigens. Ipilimumab is an FDA-approved immunotherapy for stage IV melanoma.<sup>7</sup> It is a monoclonal antibody that blocks cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), which normally acts as an immune checkpoint in the body that inhibits T cell activation. Therefore, by targeting CTLA-4, ipilimumab enhances T cell activation and cytokine production. In a phase 3 randomized controlled study, ipilimumab

significantly improved overall survival in metastatic melanoma.<sup>8</sup>

Data suggested a 28% to 34% decrease in mortality rates in advanced melanoma patients who were treated with ipilimumab and a significant improvement in overall survival.<sup>7</sup> However, drawbacks can include a potential vast and dangerous array of autoimmune side effects, including but not limited to: enterocolitis, hypophysitis, pancreatitis, leukopenia, hepatitis, and these drug toxicities can be treatment-limiting and life threatening.<sup>8,9</sup>

***PD-1 and PD-L1 Inhibitors***

Programmed cell death protein 1 (PD-1) is a receptor expressed on lymphocytes that binds its ligand PD-L1 expressed on tumor cells. The PD-1/PD-L1 is an immune checkpoint interaction that ultimately leads to T cell exhaustion and tumor cell evasion.<sup>10</sup> Nivolumab is a monoclonal antibody developed to target PD-1, inhibiting its interaction with PD-L1, and potentiating immune responses against tumor cells.<sup>11</sup> Clinical trials have shown promising results of nivolumab compared to other therapies<sup>11</sup>, and the efficacy of nivolumab combined with ipilimumab in a phase 3 clinical trial has shown significant regression when the two immune checkpoint blockers were combined when compared to either drug alone.<sup>4</sup>

***Chemotherapy***

Dacarbazine (DTIC) is the only FDA approved chemotherapy drug for the treatment of advanced melanoma. DTIC is believed to be an alkylating agent that adds methyl adducts onto DNA, inducing cytotoxic and antitumor effects.<sup>12</sup> However, responses to it are low at 5-12%, the responses to the treatment themselves do not last long, and it has not been proven to prolong overall survival.<sup>13,14</sup>

Table 1. Overview of currently available immunotherapy and chemotherapy options for advanced melanoma

FDA approved immunotherapy and chemotherapy for stage III and IV melanoma		
Drug	Mechanism of Action	Common Side Effects
<b>Interferon alpha-2b</b>	Increases macrophage phagocytosis, increases cytotoxicity of lymphocytes, and blocks oncogene expression	Flu-like symptoms, elevated transaminases, nausea, vomiting, diarrhea, neutropenia, leukopenia, thrombocytopenia
<b>Interleukin-2</b>	T cell growth factor	Flu-like symptoms, hypotension, arrhythmias, nausea, vomiting, diarrhea, oliguria
<b>Ipilimumab</b>	Blocks CTLA-4 immune checkpoint inhibition, causing enhanced T cell activation and cytokine production.	Rash, nausea, diarrhea, fatigue, weight loss
<b>Nivolumab</b>	Blocks PD-1 and PD-L1 immune checkpoint interaction between tumor and T lymphocyte, ultimately preventing tumor cell evasion.	Fatigue, malaise, hyperglycemia, hypertriglyceridemia, hyponatremia, lymphocytopenia.

**CURRENT FDA APPROVED TARGETED  
DRUG TREATMENTS FOR MELANOMA  
(Table 2)**

***BRAF Inhibitors***

The BRAFV600 somatic missense mutation is in approximately 66% of malignant melanomas.<sup>15</sup> The mutation entails a constitutively active RAS-RAF-mitogen-activated protein kinase (MAPK) pathway, a pathway that is in charge of cell growth and proliferation. BRAF inhibitors that are FDA approved for unresectable stage III melanoma and stage IV melanoma harboring a BRAFV600 mutation include vemurafenib and dabrafenib. Vemurafenib's initial efficacy and low toxicity profile were documented in a 2011 phase 3 randomized clinical trial by Chapman et al comparing vemurafenib to DTIC in patients harboring a BRAFV600 mutation. In the study, the overall survival of patients receiving vemurafenib compared to dacarbazine was 84% at 6 months with a relative risk of death reduction of 63%.<sup>16</sup> Another BRAF inhibitor, dabrafenib, was approved in 2013, and it showed similar results to vemurafenib.<sup>17</sup>

Due to mechanisms of resistance, the longevity of efficacy of a BRAF inhibitor has been called into question. The response duration to treatment ranges from 2 months to more than 18 months.<sup>18</sup> Researchers have shown that melanoma has a diverse array of mechanisms of resistance to escape BRAF inhibitor drug therapy. In a study by Shi et al examining acquired resistance to melanoma BRAF inhibitor therapy, multiple mechanisms of resistance were detected from tumor samples from patients: an estimated 52% of melanomas escaped via MAPK reactivating mechanisms, 4% escaped via the

phosphatidylinositol 3'-kinase (PI3K)-PTEN-AKT pathway, 18% escaped via both core pathways, and 26% of melanomas escaped in an unknown fashion. Additionally, when they were able to take samples from multiple tumors in the same patient, they discovered 81% (13/16) patients harbored multiple mechanisms of resistance.<sup>19</sup>

***MEK + BRAF Combination Inhibitors***

In order to further improve survival rates in melanoma patients harboring a BRAFV600 mutation, combination therapy targeting other aspects of the RAS-RAF-MAPK pathways have been believed to be a potential method to overcome escape pathways of melanoma cells to BRAF inhibitor monotherapy. Trametinib is an FDA approved targeted therapy option for patients who carry the BRAFV600E who have unresectable stage III melanoma or stage IV melanoma. Trametinib targets MEK, a protein downstream of BRAF. A phase 3 clinical trial showed the benefits of combining a BRAF inhibitor (dabrafenib) with the MEK inhibitor, trametinib. Robert et al's results show that combining dabrafenib with trametinib compared to vemurafenib alone yielded positive outcomes: overall survival rate at 12 months was 72% in combination treatment compared to 65% in vemurafenib alone.<sup>20</sup> There are still patients who fail combination therapy though, and when they do fail they form a more aggressive, metastatic melanoma.<sup>21</sup> Additionally, in many cases of combined MEK-BRAF inhibitor treatment, approximately 30% of patients still have signs of progressive disease at 6 months.<sup>22</sup>

Table 2. Overview of currently available targeted therapy for advanced melanoma

FDA approved targeted therapy for stage III and IV melanoma		
Drug	Mechanism of Action	Common Side Effects
<b>Vemurafenib</b>	Targets BRAFV600 mutation in MAPK pathway	Fatigue, prolonged Q-T interval, hypertension, peripheral neuropathy, rash, alopecia, skin photosensitivity, arthralgia, keratoacanthoma,
<b>Dabrafenib</b>	Targets BRAFV600 mutation in MAPK pathway	Hyperglycemia, skin rash, fatigue, headache, lymphocytopenia, arthralgia
<b>Trametinib</b>	Targets MEK in patients with a BRAFV600 mutation, a protein downstream to BRAF in the MAPK pathway	Rash, hypoalbuminemia, diarrhea, anemia, elevated transaminases

Table 3. Important melanoma signaling pathways

Signaling pathways that are potential targets for future melanoma therapies	
Pathway	Involvement in Melanoma
<b>KIT</b>	A receptor that can be targeted upstream of the MAPK pathway
<b>PI3K/AKT</b>	A pathway involved in tumor angiogenesis and invasion.
<b>Notch</b>	Embryonic cell pathway that possibly promotes tumor chemoresistance, tumorigenesis and progression
<b>WNT</b>	Related to melanoma tumor formation and metastasis
<b>Hippo</b>	A pathway thought to contribute to melanoma invasion and metastasis.
<b>Hedgehog</b>	Involved in spatiotemporal development in embryos as well as melanoma cell proliferation and metastasis.

## THE PROMISING FUTURE OF MELANOMA THERAPY (Table 3)

### ***KIT Inhibitors***

KIT is a transmembrane receptor tyrosine kinase upstream of the RAS-BRAF-MAPK pathway, and is in charge of cell survival and proliferation in melanocytes.<sup>23</sup> Many melanomas on mucosal, acral, and sun damaged sites harbor KIT mutations.<sup>24</sup> Thus, KIT inhibitors like imatinib can be a possible drug therapy. Guo et al's phase 2 trial of imatinib on KIT mutations in metastatic melanoma showed that imatinib demonstrated significant activity against patients with a KIT mutation.<sup>25</sup> However, they do note imatinib's overall response rate (23.3%) is much lower than that of vemurafenib, which had an 81% response rate in BRAF patients, indicating the KIT inhibitor has a lower specificity. In another study of imatinib treatment of metastatic melanoma, challenges in identifying appropriate patients for KIT inhibition treatment was highlighted. Compared to BRAF mutations, KIT mutations can be more widely distributed in the coding region, providing patient identification challenges in KIT inhibitor therapy.<sup>24</sup> Thus, although KIT inhibitors have had meaningful treatment outcomes in melanoma, more research is required to enhance responsiveness to this targeted therapy's overall response rate.

### ***PI3K/AKT Pathway***

The PI3K (phosphatidylinositol 3 kinase)-AKT-mTOR pathway is involved in cell proliferation, invasion, metabolism, and angiogenesis in normal and tumor cells.<sup>26</sup> It is separate from the RAS-RAF-MAPK pathway. There are studies now elucidating the relationship between the MAPK and PI3K pathways and mechanisms of tumor resistance to targeted drug therapy. In patient cell lines who were resistant to a RAF or MEK inhibitor, the PI3K pathway

was found to be frequently upregulated or persistent in response.<sup>26</sup> Thus, co-targeting the PI3K and BRAF pathways can potentially enhance metastatic melanoma sensitivity and prevent drug resistance. So far, co-targeting of MEK and PI3K/mTOR pathways showed more effective inhibition of BRAF mutant melanoma cell lines compared to the BRAF and PI3K/mTOR co-targeting inhibition.<sup>27</sup> More testing and in a wider number and range of patients is required to further elucidate the relationship.

Another protein involved in the PI3K pathway is phosphatase and tensin homolog deleted in from chromosome 10 (PTEN), a tumor suppressor gene. PTEN is a PI3K pathway inhibitor, and its loss has been associated with tumor development in 30-50% of melanomas.<sup>28,29</sup> In Stahl et al's study of mice models, PTEN loss was associated with decreased apoptosis in melanoma cells and PTEN expression associated with increased apoptosis.<sup>30</sup> Furthermore, there have been studies that show that BRAF mutant melanoma cells actually utilize PTEN loss in order to progress to metastatic melanoma. The combination of BRAF mutation and loss of PTEN is estimated in approximately 20% of melanomas.<sup>31</sup> Thus, a more intricate understanding of PTEN expression, PI3K pathway, MAPK and their role in melanoma tumorigenesis will be useful for combating resistance.

### ***Notch Pathway***

The notch pathway is a new avenue in battling melanoma which holds promise in preventing chemoresistance. The notch pathway is an evolutionarily conserved embryonic cell pathway important for cell fate and differentiation.<sup>32</sup> Studies have suggested that dysregulated notch signaling can prolong and confer life to cancer stem cells, which are thought to be important in tumor chemoresistance.<sup>33</sup> Other studies

have shown that notch activation promotes melanoma tumorigenesis and progression.<sup>32</sup> Thus, therapeutic benefit of targeting notch signaling includes preventing tumor angiogenesis, cancer stem cell depletion, and cell death.<sup>34</sup> The notch receptor family consists of four transmembrane receptors (Notch 1-4).<sup>35</sup> Recent research on melanoma cells correlate metastasis of melanoma cells with expression of NOTCH4 gene, suggesting this protein and the notch pathway can be a potential target of melanoma therapy.<sup>36</sup> There is currently research on drug therapy that can target notch signaling in melanoma cells. Kaushik et al have demonstrated that Honokiol (a biphenolic organic compound) can target notch signaling, confirmed with decreased downstream effector target genes of notch: Hes-1, and cyclin D1.<sup>35</sup>

### **WNT Signaling Pathway**

The WNT signaling pathway is important for cell proliferation, differentiation, migration, and many other processes of cell fate during embryonic development.<sup>37</sup> Beta-catenin is a downstream transcription factor of the WNT pathway that can translocate to the nucleus for target gene expression. Understanding of the WNT signaling pathway in melanoma progression may be critical-studies have found canonical and noncanonical WNT signaling effect different stages of tumor progression, with canonical affecting melanoma formation and noncanonical affecting melanoma metastasis.<sup>38</sup> There exists controversy in regards to the exact involvement WNT signaling has on melanoma behavior. One study showed loss of nuclear beta-catenin staining was associated with aggressive melanoma behavior,<sup>39</sup> and another study showed that elevated levels of nuclear beta-catenin was associated with reduced proliferation of melanoma cells.<sup>40</sup> Combined these findings suggest that Wnt/beta-catenin signaling is

important for melanoma cell homeostasis, and if dysregulated, can lead to transformation of melanoma cells.<sup>41</sup>

### **Hippo Signaling**

While many drug therapies and studies are focusing on the RAS-RAF-MAPK pathway in melanoma, fewer studies are focusing on targeting the invasive potential of melanoma cells. The mechanisms underlying melanoma invasion are presently poorly understood. The Hippo pathway (the Salvador-Warts-Hippo) is an evolutionarily conserved mechanism in charge of tissue and cell growth.<sup>42</sup> Downstream in the Hippo pathway are YAP and TAZ effector proteins which are amplified in many cancers and promote epithelial-mesenchymal transition-a frequent hallmark of metastasis.<sup>43</sup> In a model of skin reconstruct, YAP overexpression led to increased melanoma cell invasiveness, and YAP knockdown led to decreased metastasis potential.<sup>43</sup> Thus, mutations in the Hippo pathway yield increased activation of YAP and TAZ, which promote metastasis regardless of BRAF mutation status in melanomas.<sup>42,44</sup> Current research is examining verteporfin,<sup>44</sup> a molecule that inhibits YAP function and whether or not it can successfully regulate Hippo effector functions and subsequently melanoma invasion.

### **Hedgehog Pathway**

Another way to target cancer stem cells involves the hedgehog signaling pathway. This is an evolutionarily conserved pathway in charge of spatiotemporal development in embryos.<sup>45</sup> The pathway includes Smoothed G-protein coupled receptor-like receptor (SMO)<sup>45</sup> and downstream of SMO include many transcription factors including GLI1 and GLI2, both proven in recent years to help melanoma cell proliferation and metastasis.<sup>46,47</sup> Microarray gene expression profiles of metastatic melanoma tumors

showed elevated SMO, which correlated with overall decreased survival in melanoma patients.<sup>45</sup> Promising studies have demonstrated that by selectively blocking SMO in the Hedgehog pathway with NVP-LDE-225, melanoma growth is suppressed in vitro and in vivo.<sup>45,48</sup>

## CONCLUSION

Melanoma is a heterogenous tumor capable of resisting drugs through escape mechanisms. Existing FDA approved monotherapies have mostly been unsuccessful in the treatment of metastatic melanoma. Emerging research targeting embryonically conserved pathways, among others, are showing promising solutions to beating this cancer.

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