Vemurafenib and ipilimumab have improved overall survival in patients with metastatic melanoma.

New Targeted Therapies in Melanoma
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Background: The previous 2 years have been an exciting time in melanoma research, due in part to the approval of vemurafenib and ipilimumab for advanced melanoma. Increased knowledge of the molecular biology leading to melanoma has led to the development of several new agents that target specific oncogenes.

Methods: The authors review the latest developments in signal transduction inhibitors and in immune modulators for the treatment of melanoma. Investigational agents currently in development are also discussed.

Results: Vemurafenib and ipilimumab have improved overall survival in patients with metastatic melanoma. Many new agents are in development, including programmed death-1 antibodies and combination signal transduction inhibitors.

Conclusions: A recognition of the genetic diversity of melanoma and a better understanding of the immune system have resulted in improvements in overall survival in patients with metastatic melanoma. Refractory cases remain challenging, and combination therapies are being explored in an effort to overcome resistance mechanisms. New molecular targets need to be identified to help the subset of patients who do not harbor BRAF mutations.

Introduction
The worldwide incidence of melanoma has increased over the past few decades, with more than 132,000 people diagnosed with the disease every year. In the United States, nearly 9,500 individuals will die of melanoma in 2013. Surgery has been the mainstay of treatment for localized disease. However, despite adequate surgery and approved adjuvant treatments, recurrence rates remain high for stage III disease, with relapse-free survival rates of 63%, 32%, and 11% for stages IIIA, IIIB, and IIIC, respectively. The prognosis for patients with stage IV disease is poor, with a historical median survival of approximately 9 months.

Until recently, no systemic therapy in randomized trials demonstrated an improvement in median survival benefit for patients with metastatic melanoma. The alkylating chemotherapeutic agent dacarbazine was approved in the United States in the 1970s and demonstrated response rates of approximately 15%. Randomized trials have shown that long-term durable responses were achieved in about 5% of patients using the immunotherapy agent interleukin 2 (IL-2).
However, the low response rates and significant toxicities preclude this treatment in the majority of patients with melanoma. The identification of specific oncogenic-driving mutations and the evolving knowledge of the immune biology of melanoma have led to considerable advances in the treatment of metastatic melanoma. The use of agents such as vemurafenib and ipilimumab has significantly improved the survival rates of patients with metastatic melanoma. New agents are being developed to further advance the field and overcome the limitations of the current therapeutic agents.

In this review, we examine the various molecular pathways paramount in the development of drugs for melanoma, and we also discuss recent advances in drug development. Particular attention is made to emerging strategies to improve outcomes for patients with stage IV melanoma.

**Biology of BRAF**

The discovery in 2002 of activating mutations in the serine/threonine kinase gene *BRAF* in approximately 50% of melanomas led to efforts to develop agents to block this kinase. An understanding of the rat sarcoma (RAS)/rapidly accelerated fibrosarcoma/mitogen-activated protein kinase (MEK/MAPK)/extracellular signal-regulated kinase signal transduction pathway was important for the development of drugs to fight melanoma. Mutations have been described at a number of sites in the *BRAF* gene. All mutations lead to constitutive activity of the MAPK pathway without the need for upstream activation signals, thus resulting in increased proliferation. Approximately 80% of mutations result from the substitution of glutamic acid (E) for valine (V) in codon 600, the *BRAF* V600E mutation. Other common *BRAF* mutations in melanoma are V600K (about 16% of mutations) and V600D/R (3% of all mutations). These less common variants are found at slightly higher rates in melanomas arising in older patients. A total of 80% of benign and dysplastic nevi harbor the mutation, leading researchers to conclude that the *BRAF* mutation is necessary for melanoma development but by itself does not cause melanoma development.

**Drug Development of V-Raf Murine Sarcoma Viral Oncogene Homolog B1 Inhibitors in Melanoma**

Sorafenib was the first drug studied to target the *BRAF*-mutated melanomas. Despite some activity in cell culture and melanoma xenograft models, sorafenib ultimately showed little activity in patients with melanoma, regardless of tumor mutations status. Newer agents are more potent inhibitors of mutant v-raf murine sarcoma viral oncogene homolog B1 (BRAF) protein than sorafenib, a multikinase inhibitor. Of these newer agents, vemurafenib and dabrafenib have been the most evaluated.

The development of vemurafenib, a potent BRAF inhibitor, gave promise to the field of BRAF inhibition in melanoma. In a phase I/II clinical trial, approximately 80% of patients with stage IV *BRAF* V600E-mutant melanoma treated at the recommended phase II oral dose of 960 mg twice daily responded to vemurafenib. The 1- and 2-year survival rates for the phase I trial data in these patients were approximately 50% and 38%, respectively, with a median overall survival (OS) of 13.8 months.

A phase II trial of twice-daily vemurafenib 960 mg enrolled 132 patients with stage IV melanoma who had progressed on other therapies. The objective response rate was 53%, with 6% of patients achieving complete responses. Median OS was 15.9 months and the median progression-free survival (PFS) for this refractory population was 6.8 months. A phase III trial randomized 675 untreated patients with unresectable stage III or stage IV *BRAF* V600E-mutant melanoma to either vemurafenib (oral 960 mg twice daily) or dacarbazine chemotherapy. This study was stopped early in December 2010 at the recommendation of the Data Safety Monitoring Board at the first planned interim analysis. Patients randomized to dacarbazine were allowed to cross over to vemurafenib because of a statistically significant OS benefit for vemurafenib over dacarbazine on interim analysis. The objective response rate with vemurafenib was 48%, with a median PFS of 5.3 months compared with a 5% response rate and median PFS of 1.6 months with dacarbazine.

Updated OS data for this trial demonstrated a median OS of 13.6 months in patients receiving vemurafenib and 9.7 months in those receiving dacarbazine. Vemurafenib was approved by the US Food and Drug Administration for unresectable or metastatic *BRAF*-mutant melanoma in late 2011.

Dabrafenib is another potent BRAF inhibitor studied in patients with *BRAF*-mutant metastatic melanoma. In a phase II study of 92 patients with melanoma, 16 patients had the V600K mutation. Objective response rates of 59% in the V600E cohort and 12.5% in the V600K cohort were reported. The median PFS rates were 27 and 20 weeks, respectively, in the two mutation cohorts. The activity of dabrafenib was confirmed in a phase III trial that randomized patients with metastatic melanoma who were treatment-naive to dabrafenib at 150 mg twice daily or dacarbazine. Patients receiving dabrafenib had a PFS of 5.1 months compared with 2.7 months in those receiving dacarbazine.

**BRAF Inhibitor Resistance**

Both intrinsic and acquired resistance occurs following treatment with BRAF inhibitors, with some pa-
patients not responding to these agents while others have a short-lived response. Multiple genetic changes within a given melanoma may contribute to this phenomenon, and research is currently ongoing to further clarify patterns of resistance. Both phosphatase and tensin homolog (PTEN) and cyclin D1 have been implicated in mechanisms of intrinsic resistance. Patients whose tumors have both \textit{BRAF} mutations and PTEN dysfunction have a lower response rate to dabrafenib. In addition, cell lines with both cyclin D1 amplification as well as \textit{BRAF} mutation do not undergo apoptosis when exposed to BRAF inhibitors.

Given that the PFS rate is approximately 6 months for patients treated with drugs such as vemurafenib, it is clear that acquired resistance to BRAF inhibitors occurs in the majority of patients treated. Initially, resistance was thought to develop via mutations in the kinase that prevented drug binding; however, this is not the case. Rather, a multitude of mechanisms lead to resistance. Activation in mitogen-activated protein kinase 8 or platelet-derived growth factor receptor, beta polypeptide, as well as acquisition of \textit{NRAS} and \textit{MEK1} mutations, have been implicated in acquired resistance (Fig 1).

Squamous cell carcinomas that develop following treatment with vemurafenib have offered insight into resistance mechanisms. Studies have shown that \textit{RAS} mutations are present in approximately 60% of squamous cell carcinoma cases in patients treated with vemurafenib, suggesting that preexisting mutations may predispose patients to developing these secondary malignancies. Through these mutations, a paradoxical activation of the MAPK pathway is seen when exposed to a BRAF inhibitor.

\textbf{Overcoming Resistance}

Researchers who work to combine therapies to block multiple activated pathways in melanoma may overcome the intrinsic and acquired resistance to BRAF inhibitors. Given that the reactivation of the MAPK pathway is responsible for the majority of resistance, it is logical to combine BRAF inhibitors with MEK inhibitors. A study to assess dabrafenib/trametinib demonstrated an improvement in PFS. The median PFS was 9.4 months for patients treated with dabrafenib/trametinib compared with a PFS of 5.8 months in patients treated with dabrafenib alone. An improved survival rate was seen with trametinib compared with chemotherapy in patients with \textit{BRAF}-mutant metastatic melanoma: median PFS was 4.8 months with trametinib and 1.5 months with chemotherapy.

Vemurafenib has also been combined with the MEK inhibitor GDC-0973 for patients with metastatic melanoma. Early-phase studies have shown preliminary efficacy and tolerability of this combination. A phase III study is planned of this combination.

Clinical trials are ongoing to study other combinations, including alternative MEK inhibitors, phosphatidylinositol 3-kinase (PI3K) inhibitors, and heat shock protein 90 inhibitors combined with BRAF inhibitors.

\textbf{NRAS}

\textit{NRAS} mutations are present in approximately 15% to 20% of cutaneous melanomas. Because tumors with \textit{NRAS} mutations rarely, if ever, harbor \textit{BRAF} mutations, they may represent a distinct subpopulation.

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\textbf{Fig 1. — Mechanism of \textit{BRAF} resistance.} Akt = protein kinase B, ERK = extracellular signal-regulated kinase, MAP3K8 = mitogen-activated protein kinase cascade, MEK (MAPK) = mitogen-activated protein kinase, PI3K = phosphoinositol-3 kinase, RTK = receptor tyrosine kinase.
for potential targeted therapy, with approximately 40% of \textit{BRAF} wild-type melanomas harboring a mutation in \textit{NRAS}.\textsuperscript{29} Drug development in this subset of melanoma has been difficult as multiple pathways are unregulated by this mutation. Studies are currently evaluating the role of MEK inhibitors in this population of patients (NCT00866177, NCT01320085). MEK162 achieved responses in more than 20% of the 28 treated patients with mutant \textit{NRAS}.	extsuperscript{30} However, results with trametinib are not as promising, with no objective clinical response seen in 9 patients with \textit{NRAS} mutation.	extsuperscript{31}

Also seen in this population of patients with melanoma is the activation of PI3K signaling.\textsuperscript{32} Available data regarding the efficacy of combining PI3K inhibition with MEK inhibition are limited, but studies are ongoing.

Targeting the cell cycle via cyclin-dependent kinase (CDK) inhibitors in \textit{NRAS}-mutant melanoma is another area of research. CDK4 has been implicated in mouse models in resistance to MEK inhibitors.\textsuperscript{33} A CDK4/6 inhibitor (PD-032991) demonstrated antitumor activity in melanoma.\textsuperscript{34} CDK4/6 inhibitors for melanoma are currently being studied in clinical trials.

\textbf{Type III Transmembrane Receptor Tyrosine Kinase Alterations}

Activating \textit{KIT} mutations may be present in acral lentiginous melanosomas and mucosal melanomas, with about 10% to 20% of cases harboring this alteration.\textsuperscript{35} Imatinib mesylate, a multikinase inhibitor targeting Abl, type III transmembrane receptor tyrosine kinase (KIT), and platelet-derived growth factor receptor, has been studied in this population of patients with melanoma. In a phase II trial, 43 patients with metastatic melanoma harboring \textit{KIT} mutations or amplifications were treated with imatinib at 400 mg daily.\textsuperscript{36} The response rate was 23.3% and the median PFS was 3.5 months, and 9 of the 10 responders had tumors with mutations in exon 11 or 13. The patients with \textit{KIT} amplification without an activating mutation derived little benefit from imatinib therapy. Thus, targeting \textit{KIT} has not had the effect originally predicted for this group of patients.\textsuperscript{37,38}

\textbf{Mutations in Uveal Melanomas}

Uveal melanomas are molecularly distinct from other types of melanoma, although MAPK activation is still present. Approximately one-half of uveal melanomas harbor mutations in \textit{GNAQ}, while many others possess a mutation in \textit{GNA11}.\textsuperscript{39} Both of these mutations lead to the upregulation of the MAPK pathway. Targeted agents against MEK are under review in this population. Isolated responses to the MEK inhibitor selumetinib have been reported.\textsuperscript{40,41} A larger study with this agent in uveal melanoma is ongoing, and preliminary data are expected in the near future (NCT01143402).

\textbf{Immunotherapy}

Melanoma is often targeted for immune therapies on the basis of case reports of spontaneous regression of tumor. For many years, high-dose IL-2 was the only approved immunotherapy for stage IV melanoma, but its acute toxicities and low overall response rates (10% to 15%) have limited its use.\textsuperscript{42} Research has concentrated on improving immunotherapy for the treatment of melanoma. Ipilimumab was recently approved and demonstrated an OS benefit in randomized trials; however, overall response rates are still low. Both high-dose IL-2 and ipilimumab are associated with long-term durable responses. Study results have shown that 20% to 30% of patients receiving ipilimumab achieve disease control 3 to 4 years following treatment.\textsuperscript{43-45} Combination immunotherapy studies are also underway.

\textbf{Cytotoxic T-Lymphocyte Antigen-4 Antibodies}

Immune-modulating antibodies came to the forefront with ipilimumab, which is a fully human monoclonal antibody against cytotoxic T-lymphocyte antigen-4 (CTLA-4). CTLA-4 binds to B7 on antigen-presenting cells and downregulates the T-cell response in an activated T cell. The CTLA-4 blockade via ipilimumab continually activates the T cell (Fig 2). Ipilimumab was approved by the US Food and Drug Administration in 2011 following the publication of results of a pivotal phase III trial of ipilimumab given at 3 mg/kg.\textsuperscript{44} That study showed that ipilimumab improved OS rates in patients with metastatic, treatment-refractory melanoma compared with vaccination with gp-100 peptides. The median OS was 10 months compared with 6.4 months in the vaccine-only arm. Response rates to ipilimumab were low (approximately 15%) despite the OS advantage, thus highlighting the kinetics of this agent. BRAF inhibitors and chemotherapy may lead to rapid, short-lived responses. This is in contrast to the slow but long-term response seen with ipilimumab, with the average response taking between 3 to 6 months to be seen.

Ipilimumab has also been studied in combination therapies. The most prominent study evaluated ipilimumab at 10 mg/kg with dacarbazine compared with dacarbazine alone in patients with metastatic melanoma who were treatment-naive.\textsuperscript{42} The results were consistent with the previous trial, with approximately 20% of patients alive 4 years following treatment compared with approximately 10% of patients alive 4 years following treatment with dacarbazine alone. Ipilimumab has also been combined with the oral derivative of dacarbazine, temozolomide, in a phase II trial.\textsuperscript{46,47} Study results demonstrated a 31%
response rate in 64 patients treated, with a median PFS of 22 weeks.

Bevacizumab, a vascular endothelial growth factor inhibitor, has been combined with ipilimumab in a small study. Among the 21 patients treated, 8 achieved partial responses and 6 had stable disease. Further study of this combination is being explored.

Another combination with promising preliminary data is fotemustine/ipilimumab. A phase II study evaluated this combination in 86 patients. The immune-related disease control rate was approximately 50%, and patients with brain metastases had a similar response. The 1-year OS rate was more than 50% for all patients. A randomized phase III trial will explore this combination further in patients with advanced melanoma metastatic to the brain (NCT01654692).

One clear combination for further exploration is CTLA-4 antibodies with BRAF inhibitors. The combination is logical, given the lack of long-term benefit with vemurafenib and the lack of significant response rates with ipilimumab. The ongoing phase I/II study of CA184-161 explored this issue with ipilimumab/vemurafenib but was prematurely closed secondary to unacceptable toxicity (NCT01400451). The survival benefit of ipilimumab was dampened by the 15% to 20% incidence of clinically significant autoimmune events. Colitis, dermatitis, hepatitis, and endocrinopathies are at the forefront of these events; therefore, the careful management of these immune-related adverse events is critical for the safe and effective use of this agent.

**PD-1 Antibodies**

The programmed death 1 (PD-1) is a negative regulator of T cells. Unlike CTLA-4, PD-1 receptor ligand (PD-L1) is directly expressed by tumor cells. When PD-L1 binds to the PD-1 receptor, there is a downregulation of the T cells (Fig 3). PD-1 and PD-L1 antibodies directly activate cancer-specific T cells. Nivolumab...
is a fully human PD-1 antibody being explored in melanoma, lung, and renal cancers, demonstrating an approximately 30% objective response rate in melanoma. Similar immune toxicities were seen with these agents but at a lesser rate and reduced severity. Interstitial pneumonia is the most serious immune complication of this agent, with deaths resulting from this complication. The PD-L1 antibody MDX-1105 demonstrated objective responses in melanoma. Thus far, both the response and toxicity rates were lower than those reported with PD-1 antibodies.

Conclusions

Progress has been made with molecularly targeted agents and immune-targeted therapy for the improved survival rates in patients with metastatic melanoma. However, long-term survivors of stage IV melanoma are still the exception rather than the rule, and the majority of patients who respond to targeted therapies eventually develop resistance and disease progression. Combinations of targeted therapies are required and are being studied to prevent or delay resistance as well as to further improve survival rates for this patient population. In addition, alternative molecular targets need to be identified, particularly for patients not harboring BRAF mutations. Major advances have been made in immunotherapy for melanoma. Ipilimumab results in durable responses in a relatively small subset of patients. Significant excitement exists surrounding the development of programmed death-1 antibodies. Further study of patterns of resistance to both immunologic and targeted drugs is paramount to future drug development.

References


15. Chapman PB, Hauschild A, Robert C, et al. Updated overall survival (OS) results for BRIM-3, a phase III randomized, open-label, multicenter trial comparing BRAF inhibitor vemurafenib ( vem) with dacarbazine (DTIC) in previously untreated patients with BRAFV600E-mutated melanoma. J Clin Oncol. 2012(suppl);8502.


