Combining Targeted Therapy With Immunotherapy in BRAF-Mutant Melanoma: Promise and Challenges

Siwen Hu-Lieskovan, Lidia Robert, Blanca Homet Moreno, and Antoni Ribas

ABSTRACT

Recent breakthroughs in the treatment of advanced melanoma are based on scientific advances in understanding oncogenic signaling and the immunobiology of this cancer. Targeted therapy can successfully block oncogenic signaling in BRAFV600E-mutant melanoma with high initial clinical responses, but relapse rates are also high. Activation of an immune response by releasing inhibitory check points can induce durable responses in a subset of patients with melanoma. These advances have driven interest in combining both modes of therapy with the goal of achieving high response rates with prolonged duration. Combining BRAF inhibitors and immunotherapy can specifically target the BRAFV600E driver mutation in the tumor cells and potentially sensitize the immune system to target tumors. However, it is becoming evident that the effects of paradoxical mitogen-activated protein kinase pathway activation by BRAF inhibitors in non–BRAF-mutant cells needs to be taken into account, which may be implicated in the problems encountered in the first clinical trial testing a combination of the BRAF inhibitor vemurafenib with ipilimumab (anti-CTLA4), with significant liver toxicities. Here, we present the concept and potential mechanisms of combinatorial activity of targeted therapy and immunotherapy, review the literature for evidence to support the combination, and discuss the potential challenges and future directions for rational conduct of clinical trials.

INTRODUCTION

Cancer immunotherapy has gained much interest because of the promise of long-term disease control, even in widely metastatic disease, highlighted by the recent success of immune checkpoint inhibitors and adoptive cell transfer (ACT) therapy in advanced melanoma.4,5 There is a relatively low response rate likely due to cancer cells that developed multiple mechanisms to evade immune surveillance and suppress effector function in the tumor microenvironment.5,6 Therefore, combinatorial strategies to improve outcome of immunotherapy have been investigated. Growing evidence has suggested that oncogene-targeted therapies not only can provide additive effects but also can sensitize the tumor cells to immune attacks and improve the effector function of immune cells. Metastatic melanoma has served as a prime example to illustrate the potential combinatorial benefits of targeted therapy and immunotherapy.

BRAF-Targeted Therapy and Cancer Immunotherapy: Two Active Strategies in Patients With Melanoma

Approximately 40% of cutaneous metastatic melanomas have an activating mutation in BRAFV600E, inducing constitutive signaling through the mitogen-activated protein kinase (MAPK) pathway, providing the signals for cancer cell proliferation and survival.7 Vemurafenib and dabrafenib, two selective BRAF inhibitors targeting this mutation, and the MEK inhibitor trametinib, have been shown in phase III clinical trials to induce significant response rates and survival benefit in patients with unresectable or metastatic BRAFV600E melanoma.8-10 BRAF inhibition is associated with complications of cutaneous squamous cell carcinomas, which is an on-target adverse effect resulting from a paradoxical activation of the MAPK pathway in cells with wild-type BRAF and strong upstream signaling in the MAPK pathway11,12 and is of particular relevance when combined with immunotherapies. Despite the high response rate with BRAF inhibitors, most patients progress within a year because of acquired secondary resistance, mostly through reactivation of the MAPK pathway13-15; therefore, combined BRAF and MEK inhibition has emerged as a favorable strategy preventing these MAPK reactivation mechanisms of resistance with decreased toxicities, including a lower incidence of secondary cutaneous squamous cell carcinomas by blocking paradoxical MAPK activation.16

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0732-183X/14/3221w-2248w/$20.00
DOI: 10.1200/JCO.2013.52.1377
Melanoma responds to several modes of immunotherapy in a subset of patients, including high-dose interleukin-2 (IL-2), ACT that uses autologous tumor-infiltrating lymphocytes (TILs) or genetically engineered T cells against known melanoma tumor antigens such as MART-1 or NY-ESO-1. Ipilimumab, a monoclonal antibody that targets inhibitory immune checkpoint protein CTLA-4, was the first agent shown in phase III clinical trials to have a survival benefit in metastatic melanoma that is durable in 10% to 20% of patients. Conceivably, the main adverse effects of ipilimumab are autoimmune in nature. More recently, antibodies blocking the programmed cell death protein 1 (PD-1) or its ligand (PD-L1), another immune checkpoint pathway that limits the effector phase of a cytotoxic antitumor response, were shown in phase I trials to induce a 30% to 50% of responses in patients with melanoma, most of them being durable, with a reasonably well-tolerated toxicity profile. Furthermore, the combination of nivolumab (anti-PD-1) and ipilimumab was evaluated in a phase I trial and resulted in a response rate that seems beyond what would be achievable with either agent alone. However, a significant increase in adverse events was observed in up to 50% of patients. Identifying biomarkers to predict clinical benefit to these immunotherapies has been challenging. In one series, an increased absolute lymphocyte count after two cycles of ipilimumab was correlated with a significantly improved clinical benefit rate and median overall survival. A retrospective study of the phase I nivolumab trial suggested that PD-L1 expression level might be a predictive marker for anti-PD-1 activities, given that none of the patients without PD-L1 expression had a clinical response to nivolumab. However, because of the small cohorts and lack of standards of positive expression, these biomarkers have to be validated in prospective trials before they can be incorporated into clinical practice or before patients can be selected to use these therapies.

**How BRAF Inhibitors and Immunotherapy May Have Combinatorial Effects**


![Fig 1. Effects of BRAF inhibitors on melanoma and immune cells. Melanoma tumor cells with a BRAF\(^V600E\) mutation experience a decrease in oncogenic mitogen-activated protein kinase (MAPK) pathway signaling when treated with a BRAF inhibitor, evidenced by phosphorylation of ERK (pERK). (A) In lymphocytes, the exposure to a BRAF inhibitor instead leads to a paradoxical activation of the MAPK pathway and increased phosphorylated ERK (pERK). (B) BRAF inhibitor therapy in melanoma cells has been reported to increase melanoma antigen expression and T-cell recognition directly or indirectly. (C) Chronic exposure to BRAF inhibitors has been shown to upregulate the surface expression of the immune checkpoint ligand (PD-L1) of programmed death receptor-1 (PD-1). (D) The tumor microenvironment (macrophages, tumor-associated fibroblasts [TAFs]) can be modulated by BRAF inhibitors to enhance the immune response. IL, interleukin; PD-1, programmed death receptor-1; MDSC, myeloid-derived suppressor cell; MEK, MEK inhibitor; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; TNF-\(\alpha\), tumor necrosis factor alpha; Tyr, tyrosinase; VEGF, vascular endothelial growth factor. © 2014 by American Society of Clinical Oncology 2249.](http://www.jco.org)
immunotherapy, with the seemingly double bonus effect of BRAF inhibitors to specifically target driver mutation in the tumor cells and sensitize the immune system to target tumors. The anticipation of benefits from such a combination are based on the clinically validated individual activity of both modes of therapy, the potentials for combining without limiting overlapping toxicities, the scientific rationale of potential benefits, and the supportive evidence from preclinical models (Figs 1 and 2 and Tables 1 and 2).

**Potential Mechanisms of Combinatorial Effects**

**Increased antigen expression.** Upregulation of the expression of melanocyte differentiation antigens such as MART-1, gp100, and tyrosinase, or class I major histocompatibility complex molecule induction by BRAF-mutant melanoma cells on exposure to BRAF inhibitors have been described by several studies in both human melanoma cell lines and patients’ tumor biopsies. MEK inhibitors were also found to increase melanocyte differentiation antigen expression in both BRAF-mutant and wild-type melanoma cells. The increase in melanosomal antigen expression follows increased MITF-M levels in several cell lines, in accordance with blockade of the BRAF and MAPK activity, leading to improved antigen-specific T-cell recognition. These observations suggest that both BRAF and MEK inhibitors can increase the expression of melanoma tumor antigens, and the addition of MEK inhibition to BRAF inhibition does not compromise, and may augment, the increased antigen presentation by the tumor cells. Interestingly, in reported cases, melanoma antigen expression was significantly decreased at the time of progression on BRAF inhibitors accompanied by downregulation of MITF and was restored when subsequent combined BRAF and MEK inhibitors were given. Another possibility leading to increased tumor antigen presentation would be that tumor antigens from dying melanoma cells were picked up by host antigen-presenting cells and were cross presented to T cells. This would incriminate an immunogenic mechanism of cell death on BRAF inhibition. At this time, however, there is no experimental or human sample data to support that this is happening when using BRAF inhibitors.

**Improved lymphocyte homing and function.** An increased number of TILs was observed in early tumor biopsies of patients treated with BRAF inhibitors, in particular CD8+ (but not CD4+) T cells. The CD8+ T-cell increase has been further characterized by sequencing the highly variable complementarity-determining region 3 (CDR3) of rearranged T-cell receptor (TCR) β chain-coding genes. Data showed an increase in clonality after BRAF inhibition and a better response in those patients who had a high proportion of preexisting dominant TCR clones. This suggests that the T-cell infiltrate may not be a passive event; instead, it may be an antigen-driven recruitment into regressing tumors. On disease progression when using BRAF inhibitors, there was a decrease in TILs, which was restored with initiation of combined BRAF and MEK inhibition. Conversely, an inducible BRAF-mutated melanoma mouse model found decreased TILs after BRAF inhibitor treatment. By using a different approach with a syngeneic fully immunocompetent mouse model of melanoma, it has been demonstrated that the synergistic effect of vemurafenib and ACT was not by increase of melanoma antigen expression or TIL infiltration, but by increased function of T cells and, likely through paradoxical activation of the MAPK pathway in T cells by vemurafenib.

**Effects of BRAF and MEK Inhibition on Tumor Microenvironment**

Mutated BRAF signaling controls multiple oncogenic programs, including evasion of the immune system through the production of immune suppressive factors. BRAF blockade could decrease these immune suppressive factors and therefore improve the tumor microenvironment, making it more permissible to T-cell infiltration. Perhaps the first evidence showing a link of the MAPK pathway and immune evasion in melanoma was that MEK inhibition and RNA interference for BRAF could decrease production of the immunosuppressive soluble factors IL-6, IL-10, and vascular endothelial growth factor (VEGF). When introducing mutant BRAF into melanoma cells with wild-type BRAF, an increased IL-1 expression has been observed, which can be downregulated by BRAF inhibitors. This was shown to be partially attributable to the IL-1–induced upregulation of cyclooxygenase-2, PD-L1, and PD-L2 in melanoma tumor–associated fibroblasts, which in turn suppress the proliferation and function of melanoma-specific cytotoxic T cells.

PD-1, expressed on the surface of activated T cells, is part of an immunoglobulin superfamily shown to negatively regulate TCR signaling on engagement of its ligands (PD-L1 and/or PD-L2). This pathway can be hijacked by tumors to suppress immune control. Although healthy organs express little (if any) PD-L1, a variety of cancers express abundant levels of PD-L1. PD-L1 can be expressed constitutively by a subset of melanomas or induced in response to the exposure to T-cell–released interferons in what has been termed adaptive immune resistance. When studying tumor samples from patients treated with BRAF inhibitors with increased T-cell infiltration in postdosing biopsies, Frederick et al noticed increased expression of T-cell exhaustion markers, including TIM3, PD-1, and PD-L1 by
inhibitors and anti-PD-1 and/or anti-PD-L1 immunotherapies. Inhibition increased the intratumor ratio of CD8+ T cells to T regulatory cells and decreased immune suppressive tumor CCL2 expression, and that antitumor activity depended on the presence of CD8+ T cells and their intact function. In addition, significant antitumor activity was observed with the combination of BRAF inhibitors and anti-CCL2 or agonistic anti-CD137 antibodies, but not with anti-PD-1, anti-PD-L1, and anti-TIM3 antibodies. This is likely a result of the relative immune resistance of this particular animal model and the clone and schedule of the immunomodulatory antibodies used. Similarly, a xenograft model using a BRAFV600E-mutated human melanoma cell line transduced with gp100 and H-2Dd to allow recognition by gp100-specific pmel-1 T cells was used to assess melanocyte differentiation enhancement of immune responses by BRAF inhibitor PLX4720. It was found that administration of vemurafenib significantly increased the tumor infiltration and enhanced the antitumor

immunohistochemistry, suggesting a potential immune resistance mechanism. In vitro study of melanoma cell lines showed increased PD-L1 expression by BRAF inhibitor–resistant melanoma cells, mediated by c-JUN and STAT3 signaling, and addition of an MEK inhibitor could suppress the surface expression of PD-L1. This downregulation of PD-L1 by MEK inhibition was also observed in other tumor types, providing a rationale for combining MEK inhibitors and anti-PD-1 and/or anti-PD-L1 immunotherapies.

In the SM1 syngeneic BRAFV600E-mutant mouse model, BRAF inhibition increased the intratumor ratio of CD8+ T cells to T regulatory cells and decreased immune suppressive tumor CCL2 expression, and that antitumor activity depended on the presence of CD8+ T cells

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<td>Gray-Schoptf et al39</td>
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Abbreviations: ACT, adoptive cell transfer; BRAFi, BRAF inhibitor; CTLA-4, receptor on activated T cells; BRAFi, BRAF inhibitor; CD137, programmed cell death protein 1 ligand; TIL, tumor-infiltrating lymphocyte.

Table 1. Preclinical Studies
activity of adoptively transferred T cells in vivo. In addition, this increased TIL infiltration was primarily mediated by the ability of vemurafenib to inhibit melanoma tumor cell production of VEGF by reducing the binding of c-myc to the VEGF promoter. Furthermore, analysis of human melanoma biopsies before and during BRAF inhibitor treatment showed downregulation of VEGF consistent with the preclinical model.27

Despite the theoretical promise, the consequences of the combination of BRAF inhibitors with immunotherapies, especially the long-term effects, are uncertain. For example, the paradoxical activation of cells of BRAF inhibitors with immunotherapies, especially the long-term consequences of the combination of BRAF inhibitor and immunotherapy to improve the toxicity and potentiate the immune effector function (Fig 2). Conversely, an MEK inhibitor could dampen immune cell effector functions, thus negating the potential benefits of combination with immunotherapies. In one in vitro study,24 impaired T-cell proliferation and functions were reported with MEK inhibition, although the concentrations of MEK inhibitors tested in that study were relatively high (up to 100 \mu M). Alternatively, when combined with the BRAF inhibitors, MEK inhibitors might balance the potential overreacting effector cells to avoid exhaustion.

**Table 3. Ongoing Clinical Studies**

<table>
<thead>
<tr>
<th>Clinical Trial Name</th>
<th>NCT No.</th>
<th>Status</th>
<th>Phase</th>
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<tr>
<td>Systemic Therapy With Interferon, Interleukin-2 and BRAF Inhibitor</td>
<td>NCT01803212</td>
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<td>NCT01659151</td>
<td>Recruiting</td>
<td>II</td>
<td>Vemurafenib + lymphodepletion + ACT with TIL infusion + high-dose interleukin-2</td>
</tr>
<tr>
<td>Ph I Ipilimumab Vemurafenib Combo</td>
<td>NCT01400451</td>
<td>Active</td>
<td>I</td>
<td>Vemurafenib + ipilimumab (BMS-734016)</td>
</tr>
<tr>
<td>Combined BRAF-Targeted Therapy and Immunotherapy for Melanoma</td>
<td>NCT01754376</td>
<td>Recruiting</td>
<td>II</td>
<td>Vemurafenib + aldesleukin</td>
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<tr>
<td>Vemurafenib and White Blood Cell Therapy for Advanced Melanoma</td>
<td>NCT01585415</td>
<td>Recruiting</td>
<td>I</td>
<td>Vemurafenib + lymphodepletion + drug: Young TIL + aldesleukin</td>
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<td>Ipilimumab and Imatinib Mesylate in Advanced Cancer</td>
<td>NCT01738139</td>
<td>Recruiting</td>
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<td>A Phase 1b Open Label, Dose Escalation Study of PLX3397 in Combination With Vemurafenib in V600-mutated BRAF Melanoma</td>
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<td>Vemurafenib + PLX3397</td>
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<td>A Phase 1b Study of MPDL3280A (an Engineered Anti PD-L1 Antibody) in Combination With Vemurafenib (Zelboraf) in Patients With Previously Untreated BRAFV600-Mutation Positive Metastatic Melanoma</td>
<td>NCT01656642</td>
<td>Recruiting</td>
<td>I</td>
<td>Vemurafenib + MPDL3280A</td>
</tr>
<tr>
<td>Phase II Safety Study of Vemurafenib Followed by Ipilimumab in Subjects With V600 BRAF Mutated Advanced Melanoma</td>
<td>NCT01673854</td>
<td>Recruiting</td>
<td>II</td>
<td>Vemurafenib for 6 weeks followed by switch to ipilimumab (sequential)</td>
</tr>
</tbody>
</table>

**Abbreviations:** ACT, adoptive cell transfer; BMS, Bristol-Myers Squibb; IL-2, interleukin-2; PD-L1, programmed cell death protein 1 ligand; TIL, tumor-infiltrating lymphocyte; Young TIL, short-term cultured tumor-infiltrating lymphocytes.
of these combinations in the clinic. Despite the well-rooted rationale for combining selective BRAF inhibitors and immunotherapy to improve outcome of patients with BRAF\(^{V600E}\) mutant metastatic melanoma, there are several caveats that need to be considered. The dosing schedule of such combinations needs to be carefully studied for potential differential effects for each intervention. For example, when combining vemurafenib and anti-CD137 antibody in a syngeneic BRAF-mutant mouse model, starting BRAF inhibitor first or concurrently with immunotherapy was found to be superior.\(^{46}\) Consideration also needs to be given to the potential adverse effects of the combinations and exploration of alternative dosing regimens. For example, intermittent schedules of BRAF inhibitor–based therapy could make it more tolerable when combined with immunotherapy, decreasing the paradoxical MAPK activation, which might be the main contributor to the significant toxicity encountered in the first combination trial in the clinic.\(^{46}\) The rationale for intermittent regimens with BRAF inhibitors is further supported by preclinical data in which the development of acquired resistance to BRAF inhibitors was delayed with intermittent therapy.\(^{57}\) There is the possibility that immune cells can decrease the sensitivity of melanoma cells to BRAF inhibitors by triggering melanoma cell dedifferentiation through tumor necrosis factor alpha\(^{48}\) and decrease activity of the BRAF inhibitors with one potential mechanism of increased tumor necrosis factor alpha production.\(^{39}\) Therefore, the possibility of adverse effects including a potential decrease in response rates to the BRAF inhibitor must be considered with combination therapy using a BRAF inhibitor and immunotherapy. Finally, triple combination with the addition of an MEK inhibitor should be carefully assessed. Despite the potential adverse effects of MEK inhibitors on immune cells, MEK inhibitors could decrease toxicities from paradoxical MAPK activation with BRAF inhibitors.

In conclusion, there is a growing body of evidence to support the combinatorial approaches that merge the significant response rate of BRAF inhibitor–based targeted therapy with the unique ability of immunotherapy to achieve long-term durable responses in patients with advanced melanoma. However, the potential adverse effects of such combinations including increased autoimmune toxicities and interference with the individual antitumor activities need to be considered when translating to the clinic. The first priority in these clinical trials is to understand how the combination therapy affects the immune system and how the cancer cells are modulated. Patient-derived clinical data should be collected and analyzed in accordance with the preclinical model system to make this determination.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

Although all authors completed the disclosure declaration, the following author(s) and/or an author’s immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a “U” are those for which no compensation was received; those relationships marked with a “C” were compensated. For a detailed description of the disclosure categories, or for more information about ASCO’s conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

**Employment or Leadership Position:** None

**Consultant or Advisory Role:** Antoni Ribas, Roche Genentech (C), Amgen (C), Merck (C), GlaxoSmithKline (C), Novartis (C)

**Stock Ownership:** None

**Research Funding:** None

**Royalties, and Licenses:** None

**Other Remuneration:** None

**Administrative support:** Antoni Ribas

**Manuscript writing:** All authors

**Final approval of manuscript:** All authors

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**REFERENCES**


Glossary Terms

**NY-ESO-1**: gene coding for antigens recognized on neoplastically transformed cells T cells; also known as CTAG1B or cancer/testis antigen 1B.

**Adoptive Cell Transfer (ACT)**: the culture and expansion of T lymphocytes outside the body and then the infusion of those lymphocytes into patients for therapeutic purposes.

**CTLA4 (CD152)**: receptor on activated T cells that binds B7 molecules with a higher affinity than CD28, downregulating T-cell responses by inhibiting CD28 signaling.

**Immune Checkpoint**: immune inhibitory pathway that negatively modulates the duration and amplitude of immune responses. Examples include the CTLA-4:B7.1/B7.2 pathway, and the PD-1:PD-L1/PD-L2 pathway.

**Myeloid-Derived Suppressor Cell**: immature myeloid cells that are triggered to proliferate by chronic inflammation. These cells can suppress both innate and adaptive immune responses.

**PD-1**: programmed cell death protein 1 (CD279), a receptor expressed on the surface of activated T, B, and NK cells that negatively regulates immune responses, including autoimmune and antitumor responses.