Targeted Therapy and Immunotherapy in the Treatment of Metastatic Cutaneous Melanoma

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INTRODUCTION

The incidence of cutaneous melanoma has increased over the past 2 decades, with SEER estimates indicating that the number of new cases of melanoma diagnosed annually rose from 38,300 in 1996 to 76,000 in 2016. Among persons younger than 50 years, the incidence is higher in females, and younger women (aged 15–39 years) are especially vulnerable. Among persons older than 50, melanoma incidence in men is nearly twice that of women, in whom melanomas are often thicker and often associated with worse outcomes. Approximately 85% of melanomas are diagnosed at early stages when surgery is curative, but the lifetime probability of developing invasive disease is 3% in men and 2% in women.

Prior to the advent of effective immunotherapies and targeted therapies, melanoma was often managed with chemotherapy, which had dismal response rates and commensurately poor outcomes. Advances in the understanding of the molecular etiopathogenesis and immune escape responses of cutaneous metastatic melanoma have transformed therapeutic approaches. Specifically, improved understanding of the genetic mutations driving melanoma tumorigenesis coupled with insights into mechanisms of tumor-mediated immune evasion resulted in development of inhibitors of mitogen-activated protein kinases (MAPK; BRAF and MEK) along with inhibitors of negative regulatory immune checkpoints (cytotoxic T lymphocyte–associated antigen 4 [CTLA-4] and programmed cell death-1 [PD-1]). In this review, we discuss the role of immune therapy, targeted therapy, and combinations of these in the treatment of metastatic cutaneous melanoma. We limit the immunotherapy discussion to approved CTLA-4/PD-1 inhibitors and the targeted therapy discussion to approved BRAF/NRAS/MEK inhibitors and do not discuss non-checkpoint immunotherapies including cytokines (HD IL-2), vaccines, or adoptive T-cell approaches. Interested readers are directed to other excellent works covering these important topics.

DEVELOPMENT OF TARGETED AND NOVEL IMMUNE THERAPIES

For many years the degree of ultraviolet (UV) light exposure was considered the sole major risk factor for melanoma oncogenesis, even though its mechanism was largely unknown. However, clinical observations regarding the occurrence of melanoma on less exposed areas (trunk and limbs) in individuals with intermittent sun exposure led to the proposition that melanomas that arose in younger patients with intermittent sun exposure were distinct from melanomas that arose in older patients in association with markers of chronic sun exposure—the “divergent pathway” hypothesis. Critical to this understanding were whole-exome sequencing data from multiple groups, including The Cancer Genome Atlas, that identified patterns of mutations in oncogenic drivers that were distinct in patients with and without chronically...
sun-damaged (CSD) skin.4–7 It is now clear that based on its association with CSD skin, melanoma can be subclassified into CSD or non-CSD melanoma. CSD and non-CSD melanoma have distinct clinico-pathological characteristics and are associated with different driver mutations. CSD melanomas typically arise in older patients on sun-exposed areas (head/neck, dorsal surfaces of distal extremities) and are associated with particular driver mutations (BRAF non-V600E, NRAS, NF1, or KIT) and genetic signatures of UV-induced DNA damage (G > T [UVA] or C > T [UVB]) transitions. Conversely, non-CSD melanomas typically arise in younger (< 55 years) patients on intermittently sun-exposed areas (trunk, proximal extremities) and are associated with BRAF V600E/K driver mutations and often lack genetic signatures of UV mutagenesis.

Identification of driver mutations in components of the MAPK pathway, including BRAF and NRAS, facilitated the development of targeted inhibitors. The BRAF inhibitors vemurafenib and dabrafenib have been shown in pivotal phase 3 studies to significantly improve overall and progression-free survival in patients with metastatic melanoma compared with chemotherapy and garnered regulatory approval (vemurafenib, BRIM-3,8,9 dabrafenib, BREAK-3). Concomitant MEK and BRAF inhibition extends the duration of benefit by preventing downstream kinase activation in the MAPK pathway. Notably, concomitant MEK inhibition alters the side-effect profile of BRAF inhibitors, with reduced incidence of keratoacanthomas and cutaneous squamous cell carcinomas that are attributable to on-target, off-tumor effects of BRAF inhibitors. Combined BRAF and MEK inhibition (vemurafenib/cobimetinib and dabrafenib/trametinib) further improved overall and progression-free survival compared to single-agent BRAF inhibition in phase 3 studies (COMBI-d,11 COMBI-v,12 and coBRIM13). Although often deep, the responses seen with the use of targeted kinase inhibitors are not often durable, with the vast majority of patients progressing after 12 to 15 months of therapy.

In parallel, work primarily done in murine models of chronic viral infection uncovered the role played by co-inhibitory or co-excitatory immune checkpoints in mediating T-cell immune responses. These efforts clarified that tumor-mediated immune suppression primarily occurs through enhancement of inhibitory signals via the negative T-cell immune suppression CTLA-4 or PD-1.14,15 Blockade of negative T-cell immune checkpoints resulted in activation of the adaptive immune system, resulting in durable anti-tumor responses as demonstrated in studies of the CTLA-4 inhibitor ipilimumab (CA184-02016 and CA184-02417) and the PD-1 inhibitors nivolumab (CA209-003,18 CheckMate 037,19 and CheckMate 06620) and pembrolizumab (KEYNOTE-00131 and KEYNOTE-00622). Compared to the deep but short-lived responses seen with targeted kinase inhibitors, patients treated with CTLA-4 or PD-1 immune checkpoint blockade often developed durable responses that persisted even after completion of therapy. Combined CTLA-4 and PD-1 blockade results in greater magnitude of response with proportionately increased toxicity.23–25

IMMUNOTHERAPY

CTLA-4 AND PD-1 IMMUNE CHECKPOINT INHIBITORS

The novel success of immunotherapy in recent decades is largely attributable to improved understanding of adaptive immune physiology, specifically T-cell activation and regulation. T-cell activation requires 2 independent signaling events: it is initiated upon recognition of the antigen-MHC class II-receptor complex on antigen-presenting cells (APC), and requires a secondary co-stimulatory interaction of CD80/CD86 (B7.1/B7.2) on APCs and CD28 molecule on T-cells; without this second event, T-cells enter an anergic state.30–32 Upon successful signaling and co-stimulation, newly activated
T-cells upregulate CTLA-4, which can bind to B7 molecules with a nearly 100-fold greater affinity than CD28. Unlike CD28, CTLA-4 engagement negatively regulates T-cell activation. The opposing signals produced by CD28 and CTLA-4 are integrated by the T-cell to determine eventual response to activation, and provide a means by which T-cell activation is homeostatically regulated to prevent exaggerated physiologic immune responses. It was hypothesized that CTLA-4 blockade would permit T-cell activation, which is thwarted in the tumor microenvironment by tumor-mediated CTLA-4 engagement, thereby unleashing an anti-tumor immune response.

PD-1 is a member of the CD28 and CTLA-4 immunoglobulin super family and, similar to CTLA-4, binds activated T-cells. PD-1 has 2 ligands on activated T-cells: PD-L1 and PD-L2. PD-L1 is constitutively expressed by a variety of immune and non-immune cells, particularly in inflammatory environments including tumor microenvironments, in response to the release of inflammatory cytokines such as interferon (IFN)-γ. Conversely, PD-L2 is only minimally expressed constitutively, although its expression on immune and non-immune cells can be induced by similar cues from inflammatory microenvironments. PD-L1 and PD-L2 cross-compete for binding to PD-1, with PD-L2 exhibiting 2- to 6-fold greater relative affinity than PD-L1. PD-L1/PD-1 binding results in phosphorylation of 2 tyrosinases in the intracellular portion of PD-1, which contains immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM). PD-1 ITSM subsequently recruits either of 2 SH2-domain-containing protein tyrosine phosphatases: SHP-1 and SHP-2. SHP-2 signaling suppresses PI3K/Akt activation, down-regulates Bcl-xL, and suppresses expression of multiple transcription factors that mediate T-cell effector function including GATA-3, Eomes, and T-bet. The net effect of PD-L1/PD-1 engagement is to suppress T-cell proliferation, cytokine production, cytolytic function, and survival. Unlike CTLA-4, which primarily affects the priming phase of naive T-cell activation, PD-1 chiefly regulates the effector phase of T-cell function. Furthermore, because PD-L1/PD-L2 expression is limited to inflammatory microenvironments, the effects of PD-1 are less generalized than those of CTLA-4.

**SINGLE AGENT ACTIVITY OF CTLA-4 AND PD-1 INHIBITORS**

Ipilimumab (MDX-010) is a human IgG1 monoclonal antibody shown to inhibit CTLA-4. Early studies tested different formulations (transfectoma-derived and hybridoma-derived), doses, and schedules of ipilimumab primarily in patients with advanced refractory melanoma. Although responses were infrequent, responding patients experienced durable remissions at 1- and 2-year time points. Notably, in a foreshadowing of changes to response criteria used to evaluate these agents, several treated patients who initially had radiographically stable disease upon completion of therapy subsequently experienced a gradual decline in tumor burden.

Ipilimumab was subsequently evaluated in 2 phase 3 trials. The first study (MDX010-020/CA184-020), which involved 676 HLA-A*0201–positive patients with advanced melanoma, compared ipilimumab 3 mg/kg every 3 weeks for 4 doses either singly or in combination with gp100 vaccine with a gp100-only control arm. Ipilimumab administration resulted in objective responses in 11% of patients and improved progression-free and overall survival compared to gp100 vaccine with a gp100-only control arm. Ipilimumab monotherapy was superior to ipilimumab/gp100 combination, possibly related to timing of vaccine in relation to ipilimumab. A confirmatory study (CA184-024) compared a higher dose of ipilimumab (10 mg/kg) in combination with dacarbazine to dacarbazine monotherapy in previously untreated melanoma and was positive. Given the lack of augmented efficacy with the higher (10 mg/kg)
Metastatic Cutaneous Melanoma

Table 1. Phase 3 Studies of CTLA-4 and PD-1 Inhibitors in Advanced Melanoma

<table>
<thead>
<tr>
<th>Study/Patient Population/Primary Endpoint(s)</th>
<th>Intervention(s)</th>
<th>N</th>
<th>ORR (95% CI)</th>
<th>mPFS (mo) (95% CI)</th>
<th>mOS (mo) (95% CI)</th>
<th>Grade 3/4 AEs, %</th>
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<tr>
<td>CA184-020 (MDX010-020) *</td>
<td>Ipilimumab + gp100 10 mg/kg + dacarbazine (850 mg/m²)  + placebo</td>
<td>502</td>
<td>Ipilimumab + dacarbazine: 15.2%</td>
<td>mPFS not reported</td>
<td>Ipilimumab + dacarbazine: 11.2 (9.4–13.6)</td>
<td>Any: Ipilimumab + dacarbazine: 56.7  Dacarbazine: 27.5</td>
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<tr>
<td>Previously untreated advanced melanoma</td>
<td>Primary endpoint: OS</td>
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<td>Dacarbazine: 10.3%</td>
<td>HR for PFS (vs dacarbazine): 0.76 (P = 0.006)</td>
<td>Dacarbazine: 9.1 (7.8–10.5)</td>
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<tr>
<td>CA184-024 (MDX010-024) *</td>
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<td>HR 0.69 (95% CI 0.57–0.84)</td>
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<td>Previously untreated advanced melanoma</td>
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dose, ipilimumab received regulatory approval in 2011 for the treatment of melanoma at the lower dose: 3 mg/kg administered every 3 weeks for 4 doses (Table 1). Survival data was strikingly similar to patterns observed in prior phase 2 studies, with survival curves plateauing after 2 years at 23.5% to 28.5% of treated patients. Pooled survival data from prospective and retrospective studies of ipilimumab corroborate the plateau of 22% (26% treated; 20% untreated) reached at year 3 regardless of prior therapy or ipilimumab dose, underscoring the durability of long-term survival in ipilimumab-treated patients.47 Ipilimumab administration resulted in an unusual spectrum of toxicities including diarrhea, rash, hepatitis, and hypophysitis (termed immune-related adverse events, or irAEs) in up to a third of patients.
### Table 1. (continued)

<table>
<thead>
<tr>
<th>Study/Patient Population/Primary Endpoint(s)</th>
<th>Intervention(s)</th>
<th>N</th>
<th>ORR (95% CI)</th>
<th>mPFS (95% CI)</th>
<th>mOS (95% CI)</th>
<th>Grade 3/4 AEs, %</th>
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<tr>
<td><strong>Nivolumab</strong></td>
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| CheckMate 037<sup>19</sup> Advanced melanoma following progression on ipilimumab and BRAF inhibitor (if BRAF V600 mutated) | 2:1 randomization to:  
  • Nivolumab 3 mg/kg every 2 wk  
  • ICC: Dacarbazine 1000 mg/m² every 3 wk or carboplatin AUC 6 with paclitaxel 175 mg/m²  
  Continued until progression or unacceptable side effects | 405 | Nivolumab: 31.7% (2.3–6.5)  
  ICC: 10.6% (2.1–6.3)  
  6-mo PFS: 48% (nivolumab) vs 34% (ICC)  
  HR 0.82 | mOS not reported | Any: Nivolumab: 9.0  
  ICC: 32.0  
  Cutaneous: Nivolumab: 0.0  
  ICC: 0.0  
  GI (diarrhea/colitis): Nivolumab: < 1.0  
  ICC: 2.0  
  Anemia: Nivolumab: 1.0  
  ICC: 5.0  
  Neutropenia Nivolumab: 0.0  
  ICC: 14.0  
  Thrombocytopenia Nivolumab: 0.0  
  ICC: 6.0  
  Other (increased lipase) Nivolumab: 1.0  
  ICC: 0.0 |
| Checkmate 066<sup>20</sup> Advanced untreated melanoma, BRAF wild type | 1:1 randomization to:  
  • Nivolumab 3 mg/kg every 2 wk + placebo every 3 wk  
  • Dacarbazine 1000 mg every 3 wk + placebo every 2 wk | 418 | Nivolumab: 42.9% (3.5–10.8)  
  Dacarbazine: 14.4% (2.1–2.4)  
  HR 0.43 (95% CI 0.34–0.56) | Nivolumab: 5.1 mo (3.5–10.8)  
  Dacarbazine: 11.2 mo  
  Significant benefit in OS in nivolumab vs dacarbazine group (HR for death 0.42 [99.79% CI 0.25–0.73]) | Nivolumab: not reached  
  Dacarbazine: not reached  
  All: Nivolumab: 11.7  
  Dacarbazine: 17.6  
  Cutaneous: Nivolumab: 0.5  
  Dacarbazine: 0.0  
  GI (diarrhea/colitis): Nivolumab: 1.0  
  Dacarbazine: 0.5  
  Neutropenia Nivolumab: 0.0  
  Dacarbazine: 4.9  
  Thrombocytopenia: Nivolumab: 0.0  
  Dacarbazine: 4.9 | continued on page 31 |

Pembrolizumab and nivolumab are humanized IgG4 monoclonal antibodies that target the PD-1 receptor found on activated T cells, B cells, and myeloid cells. Both pembrolizumab and nivolumab are engineered similarly: by immunizing transgenic mice with recombinant human PD-1-Fc protein and subsequently screening murine splenic cells fused with myeloma cells for hybridomas producing antibodies reactive to PD-1-Fc. Like IgG1, the IgG4 moiety neither engages Fc receptors nor activates complement, avoiding cytotoxic effects of the antibody upon binding to the T cells that it is intended to activate. Both pembrolizumab and nivolumab bind PD-1 with high affinity and specificity, effectively inhibiting the interaction between PD-1 and ligands PD-L1 and PD-L2.

Nivolumab was first studied in a phase 1 study (CA209-003) of 296 patients with advanced cancers who received 1, 3, or 10 mg/kg administered.
### Table 1. (continued)

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<tr>
<th>Study/Patient Population/ Primary Endpoint(s)</th>
<th>Intervention(s)</th>
<th>N</th>
<th>ORR (95% CI)</th>
<th>mPFS (95% CI)</th>
<th>mOS (95% CI)</th>
<th>Grade 3/4 AEs, %</th>
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<tr>
<td><strong>Pembrolizumab</strong></td>
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<td>KEYNOTE-006</td>
<td>Advanced melanoma with 0 or 1 prior systemic therapy for advanced disease</td>
<td>834</td>
<td>Pembrolizumab 2 wk: 33.7% (3.4–6.9)</td>
<td>Pembrolizumab 2 wk: 5.5 mo (95% CI 0.47–0.83) and 0.69 (95% CI 0.52–0.9), respectively</td>
<td>Pembrolizumab (every 2 wk and 3 wk): not reached</td>
<td>Pembrolizumab 2 wk: 13.3</td>
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<td>Pembrolizumab 3 wk: 32.9%</td>
<td>Pembrolizumab 3 wk: 4.1 mo (95% CI 0.47–0.83) and 0.69 (95% CI 0.52–0.9), respectively</td>
<td>Ipilimumab 19.9</td>
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<td>Ipilimumab: 11.9%</td>
<td>Ipilimumab: 2.8 mo (95% CI 0.46–0.72) and 0.47–0.72), respectively</td>
<td>Ipilimumab 2 wk: 14.7</td>
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<td>Pembrolizumab 3 wk: 13.4</td>
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<td>Ipilimumab 14.5</td>
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<td>Fatigue: Pembrolizumab 2 wk: 20.9</td>
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<td>Ipilimumab 3 wk: 19.1</td>
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<td>Ipilimumab 15.2</td>
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<td><strong>Ipilimumab + Nivolumab</strong></td>
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<td>CheckMate 067</td>
<td>Advanced melanoma with no prior systemic therapy for advanced disease</td>
<td>945</td>
<td>Nivolumab + ipilimumab: 57.6% (95% CI 0.47–0.83)</td>
<td>Nivolumab + ipilimumab: 11.5 mo (95% CI 0.52–0.9)</td>
<td>Nivolumab + ipilimumab: 0.69 (95% CI 0.47–0.83) and 0.69 (95% CI 0.52–0.9)</td>
<td>Nivolumab + ipilimumab: 55.0</td>
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<td>Nivolumab: 16.3</td>
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<td>Ipilimumab: 27.3</td>
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<td>Cutaneous (rash/pruritus): Nivolumab + ipilimumab: 6.7</td>
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<td>Ipilimumab: 2.2</td>
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<td>GI (diarrhea): Nivolumab + ipilimumab: 9.3</td>
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<td>Ipilimumab: 6.1</td>
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<td>GI (colitis): Nivolumab + ipilimumab: 7.7</td>
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<td>Ipilimumab: 8.7</td>
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<td>GI (ALT/AST abnormalities): Nivolumab + ipilimumab: 14.4</td>
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<td>Fatigue: Nivolumab + ipilimumab: 4.2</td>
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<td>Nivolumab: 1.3</td>
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<td>Ipilimumab: 1.0</td>
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**AE** = adverse events; **CI** = confidence interval; **GI** = gastrointestinal; **HR** = hazard ratio; **ICC** = investigator’s choice chemotherapy; **mPFS** = median progression-free survival; **mOS** = median overall survival; **ORR** = objective response rate.
every 2 weeks. Histologies tested included melanoma, non–small-cell lung cancer (NSCLC), renal-cell cancer (RCC), castration-resistant prostate cancer (CRPC), and colorectal cancer (CRC). Responses were seen in melanoma and RCC and unusually in NSCLC, including in both squamous and non-squamous tumors. Objective responses were noted in 41% of the 107 melanoma patients treated at 3 mg/kg. Survival was improved, with 1- and 2-year survival rates of 62% and 43% at extended follow up.

Subsequently, nivolumab was compared to chemotherapy in a pair of phase 3 studies involving both previously untreated (Checkmate 066) and ipilimumab/BRAF inhibitor–refractory (CheckMate 037) patients. In both studies, nivolumab produced durable responses in 32% to 34% of patients and improved survival over chemotherapy. Compared to ipilimumab, the incidence of irAEs was much lower with nivolumab. The depth and magnitude of responses observed led to regulatory approval for nivolumab in both indications (untreated and ipilimumab/BRAF inhibitor–treated melanoma) in 2014. Data from both studies are summarized in Table 1.

Pembrolizumab was first evaluated in a phase 1 study of 30 patients with a variety of solid organ malignancies in which no dose-limiting toxicities were observed and no defined maximal tolerated dose was reached. Per protocol, maximal administered dose was 10 mg/kg every 2 weeks. Following startling responses including 2 complete responses of long duration, pembrolizumab was evaluated in a large phase 1 study (KEYNOTE-001) of 1260 patients that evaluated 3 doses (10 mg/kg every 2 weeks, 10 mg/kg every 3 weeks, and 2 mg/kg every 3 weeks) in separate melanoma and NSCLC substudies. Both ipilimumab-naïve and ipilimumab-treated patients were enrolled in the melanoma sub-study. Objective responses were seen in 38% of patients across all 3 dosing schedules and were similar in both ipilimumab-naïve and ipilimumab-treated patients. Similar to nivolumab, most responders experienced durable remissions.

Pembrolizumab was subsequently compared to ipilimumab in untreated patients (KEYNOTE-006) in which patients were randomly assigned to receive either ipilimumab or pembrolizumab at 1 of 2 doses: 10 mg/kg every 2 weeks and pembrolizumab 10 mg/kg every 3 weeks. Response rates were greater with pembrolizumab than ipilimumab, with commensurately greater 1-year survival rates. Rates of treatment-related adverse events requiring discontinuation of study drug were much lower with pembrolizumab than ipilimumab. This trial was instrumental in proving the superior profile of pembrolizumab over ipilimumab. The US Food and Drug Administration (FDA) granted pembrolizumab accelerated approval for second-line treatment of melanoma in 2014, and updated this to include a first-line indication in 2015 (Table 1).

**Efficacy of Combined CTLA-4 and PD-1 Inhibition**

Preclinical studies demonstrated that PD-1 blockade was more effective than CTLA-4 blockade and combination PD-1/CTLA-4 blockade was synergistic, with complete rejection of tumors in approximately half of the treated animals. This hypothesis was evaluated in a phase 1 study that explored both concurrent and sequential combinations of ipilimumab and nivolumab along with increasing doses of both agents in PD-1/CTLA-4–naïve advanced melanoma. Responses were greater in the concurrent arm (40%) than in the sequential arm (20%) across dose-levels with a small fraction of patients treated in the concurrent arm experiencing a profound reduction (80%) in tumor burden.

The superiority of ipilimumab/nivolumab combination to ipilimumab monotherapy was demonstrated in a randomized blinded phase 2 study (CheckMate 069). Of the 4 different ipilimumab/nivolumab doses explored in the phase 1 study (3 mg/kg and 0.3 mg/kg, 3 mg/kg
and 1 mg/kg, 1 mg/kg and 3 mg/kg, 3 mg/kg and 3 mg/kg), ipilimumab 3 mg/kg and nivolumab 1 mg/kg (followed by nivolumab 3 mg/kg) was compared to ipilimumab and nivolumab-matched placebo. Responses were significantly greater with dual PD-1/CTLA-4 blockade compared to CTLA-4 blockade alone (59% versus 11%). Concurrently, a 3-arm randomized phase 3 study compared the same dose of ipilimumab/nivolumab to ipilimumab and nivolumab in previously untreated advanced melanoma (CheckMate 067). Similar to CheckMate 069, CheckMate 067 demonstrated that ipilimumab/nivolumab combination resulted in more profound responses (58%) than either ipilimumab (19%) or nivolumab (44%) alone. Toxicity, primarily diarrhea, fatigue, pruritus, and rash, was considerable in the combination arm (55% grade 3/4 adverse events) and resulted in treatment discontinuation in 30% of patients. The profound and durable responses observed led to accelerated approval of ipilimumab/nivolumab combination in 2015 (Table 1).

Efforts to improve the toxicity/benefit ratio of ipilimumab/nivolumab combination have centered around studying lower doses and/or extended dosing schedules of ipilimumab, including ipilimumab 1 mg/kg every 6 or 12 weeks with nivolumab dosed at 3 mg/kg every 2 weeks or 480 mg every 4 weeks. Promising data from a first-line study in NSCLC (CheckMate 012) support the evaluation of nivolumab in combination with lower-dosed ipilimumab (1 mg/kg every 6 or 12 weeks). This approach is being tested against platinum doublet chemotherapy in a confirmatory phase 3 study in NSCLC (CheckMate 227).

**TARGETED THERAPY**

**MAPK KINASE PATHWAY IN MELANOMA TUMORIGENESIS**

The MAPK pathway mediates cellular responses to growth signals. RAF kinases are central mediators in the MAPK pathway and exert their effect primarily through MEK phosphorylation and activation following dimerization (hetero- or homo-) of RAF molecules. As a result, RAF is integral to multiple cellular processes, including transcriptional regulation, cellular differentiation, and cell proliferation. MAPK pathway activation is a common event in many cancers, primarily due to activating mutations in *BRAF* or *RAS*. Alternatively, MAPK pathway activation can occur in the absence of activating mutations in *BRAF* or *NRAS* through down-regulation of MAPK pathway inhibitory proteins (RAF-1 inhibitory protein or SPRY-2), *C-MET* overexpression, or activating mutations in non-*BRAF/NRAS* kinases including *CRAF*, *HRAS*, and *NRAS*.

Somatic point mutations in *BRAF* are frequently observed (37%–50%) in malignant melanomas and at lower frequency in a range of human cancers including NSCLC, colorectal cancer, papillary thyroid cancer, ovarian cancer, glioma, and gastrointestinal stromal tumor. *NRAS* mutations in melanoma typically occur within the activation segment of the kinase domain (exon 15). Between 80% and 90% of activating mutations result in an amino acid substitution of glutamate (E) for valine (V) at position 600: V600E. V600E mutations are true oncogenic drivers, resulting in increased kinase activity with demonstrable transformational capacity in vitro. *BRAF* mutations are usually mutually exclusive, with tumors typically containing no other driver mutations in *NRAS*, *KIT*, *NFI*, or other genes. *NRAS* mutations are less common than *BRAF* mutations, having a reported frequency of 13% to 25% in melanoma. *NRAS* mutations generally occur within the P-loop region of the G domain (exon 2), or less commonly in the switch II region of the G domain (exon 3). Most *NRAS* exon 2 mutations comprise amino acid substitutions at position 61 from glutamine (Q) to arginine (R; 35%), lysine (K; 34%) and less often to glutamate (E), leucine (L), or proline (P).
Preclinical data suggest that NRAS mutations paradoxically stimulate the MAPK pathway and thus enhance tumor growth in vitro.\(^{59,60}\)

Several important phenotypic differences distinguish NRAS from BRAF-mutated melanoma. NRAS-mutated tumors are typically associated with increasing age and CSD skin, while BRAF-mutated tumors arise in younger patients in non-CSD skin. A large population-based study suggested that NRAS-mutated melanomas were associated with mitoses and lower tumor infiltrating lymphocytes (TIL) grade, and arose in anatomic sites other than head/neck, while BRAF-mutated tumors were associated with mitoses and superficial spreading histology.\(^{61}\)

Although the lower TIL grade seen with NRAS-mutated melanomas suggests a more immuno-suppressed microenvironment and argues for poorer responses to immune therapies, clinical studies comparing responses to immunotherapies in various categories of driver mutations provide conflicting results for the prognostic role of NRAS mutations in relation to immune checkpoint blockade and other immune therapies.\(^{62-64}\)

NF1 represents the third known driver in cutaneous melanoma, with mutations reported in 12% of cases.\(^{6,7}\) NF1 encodes neurofibromin, which has GTPase activity and regulates RAS proteins; NF1 loss results in increased RAS.\(^{65}\)

Unlike BRAF or NRAS, which are usually mutually exclusive, NF1 mutations in melanoma can occur singly or in combination with either BRAF or NRAS mutations. In these settings, NF1 mutations are associated with RAS activation, MEK-dependence, and resistance to RAF inhibition.\(^{66}\)

MAPK PATHWAY INHIBITION SINGLY AND IN COMBINATION

Although multiple MEK 1/2 inhibitors (AS703026, AZD8330/ARRY-704, AZD6244, CH5126766, CI-1040, GSK1120212, PD0325901, RDEA119, and XL518) and RAF inhibitors (ARQ 680, GDC-0879, GSK2118436, PLX4032, RAF265, sorafenib, XL281/BMS-908662) were developed, the initial evaluation of MAPK pathway inhibitors in advanced human cancers began with CI-1040. Preclinical data suggested that CI-1040 potently and selectively inhibited both MEK1 and MEK2, but phase 1 and 2 human trial results were disappointing, likely because these trials were not selectively enriched for NRAS/BRAF-mutated tumors or cancers in which these oncogenic mutations were most commonly detected, such as melanoma.\(^{67,68}\)

The subsequent evaluation of selumetinib (AZD6244/ARRY-142886) in a phase 2 study was also negative. Although investigators enrolled a presumably enriched population (cutaneous melanoma), the incidence of NRAS/BRAF-mutated tumors was not ascertained to determine this, but rather assumed, which led to a discrepancy between the assumed (prestudy) and observed (on-study) proportions of BRAF/NRAS mutations that was not accounted for in power calculations.\(^{69,70}\)

Lessons learned from these earlier misadventures informed the current paradigm of targeted therapy development: (1) identification of a highly specific and potent inhibitor through high-throughput screening; (2) establishment of maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) in unselected patients; (3) confirmation of RP2D in selected tumor types enriched for target of interest; and (4) confirmatory study against standard comparator to seek regulatory approval.

Vemurafenib and dabrafenib were evaluated in this tiered fashion in phase 1 dose-finding studies comprising unselected patients, followed by phase 2 studies in advanced BRAF V600E-mutated melanoma. Both were subsequently evaluated in randomized phase 3 trials (vemurafenib, BRIM-3; dabrafenib, BREAK-3) that compared them with dacarbazine (1000 mg/m² intravenously every 3 weeks) in the treatment of advanced BRAF V600E-mutated melanoma. Response kinetics for both agents were remarkably similar: single-agent BRAF inhibitors resulted in rapid (time to response 2–3 months),
profound (approximately 50% objective responses) reductions in tumor burden that lasted 6 to 7 months. Adverse events common to both agents included rash, fatigue, and arthralgia, although clinically significant photosensitivity was more common with vemurafenib and clinically significant pyrexia was more common with dabrafenib. Class-specific adverse events included the development of cutaneous squamous-cell carcinomas and keratoacanthomas secondary to paradoxical activation of MAPK pathway signaling either through activating mutations in HRAS or mutations or amplifications in receptor tyrosine kinases upstream of BRAF, resulting in elevated levels of RAS–guanosine triphosphate complexes.71 Results of these studies resulted in regulatory approval of single-agent BRAF inhibitors for the treatment of BRAF V600E (and later V600K)—mutated melanoma (vemurafenib in 2011; dabrafenib in 2013). Details regarding trial populations, study interventions, efficacy, and adverse events are summarized in Table 2.

Responses to BRAF inhibitors are typically profound but temporary. Mechanisms of acquired resistance are diverse and include reactivation of MAPK pathway–dependent signaling (RAS activation or increased RAF expression), and development of MAPK pathway–independent signaling (COT overexpression; increased PI3K or AKT signaling) that permits bypass of inhibited BRAF signaling within the MAPK pathway.72–76 These findings suggested that upfront inhibition of both MEK and mutant BRAF may

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**Table 2. Phase 3 Studies of BRAF and MEK Inhibitors in Advanced Melanoma**

<table>
<thead>
<tr>
<th>Study/Patient Population/ Primary Endpoint(s)</th>
<th>Intervention(s)</th>
<th>N</th>
<th>ORR (95% CI)</th>
<th>mPFS (95% CI)</th>
<th>mOS (95% CI)</th>
<th>Grade 3/4 AE(s), %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vemurafenib</strong></td>
<td></td>
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<tr>
<td>BRIM39</td>
<td>1:1 randomization to:</td>
<td>675</td>
<td></td>
<td>HR 0.39 (0.33–0.47, P &lt; 0.0001)</td>
<td>HR 0.75 (0.60–0.93, P = 0.0085)</td>
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<tr>
<td>• Vemurafenib 960 mg twice daily</td>
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<td></td>
<td>5.7%</td>
<td>6.9 mo (6.2–7.0)</td>
<td>HR 0.38 (95% CI 0.32–0.46)</td>
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<tr>
<td>• Dacarbazine (1000 mg/m²) every 3 wk</td>
<td></td>
<td></td>
<td>1.5%</td>
<td>Dacarbazine: 13.3 mo (11.9–14.9)</td>
<td>HR 0.7 (95% CI 0.57–0.87)</td>
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<tr>
<td><strong>Dabrafenib</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BREAK-310</td>
<td>3:1 randomization to:</td>
<td>250</td>
<td></td>
<td>HR 0.3 (95% CI 0.18–0.51)</td>
<td>HR 0.77 (95% CI 0.52–1.13, not statistically significant)</td>
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<tr>
<td>• Dabrafenib 150 mg twice daily</td>
<td></td>
<td></td>
<td>50%</td>
<td>Dabrafenib: 4.3 mo (16.6–not reached)</td>
<td>Dabrafenib: 18.2 mo (16.6–not reached)</td>
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</tr>
<tr>
<td>• Dacarbazine 1000 mg/m² IV every 3 wk</td>
<td></td>
<td></td>
<td>7%</td>
<td>Chemotherapy: 2.7 mo</td>
<td>Chemotherapy: 15.6 mo (12.7–not reached)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HR 0.3 (95% CI 0.18–0.51)</td>
<td>HR 0.77 (95% CI 0.52–1.13, not statistically significant)</td>
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</tbody>
</table>

continued on page 36
produce more durable responses than BRAF inhibition alone. Three pivotal phase 3 studies established the superiority of combination BRAF and MEK inhibition over BRAF inhibition alone: COMBI-d\textsuperscript{11} (dabrafenib/trametinib versus dabrafenib/placebo), COMBI-v\textsuperscript{12} (dabrafenib/trametinib versus vemurafenib), and coBRIM\textsuperscript{13} (vemurafenib/cobimetinib versus vemurafenib/placebo). As expected, compared to BRAF inhibitor monotherapy, combination
## Metastatic Cutaneous Melanoma

### Table 2. (continued)

<table>
<thead>
<tr>
<th>Study/Patient Population/Primary Endpoint(s)</th>
<th>Intervention(s)</th>
<th>N</th>
<th>ORR (95% CI)</th>
<th>mPFS (95% CI)</th>
<th>mOS (95% CI)</th>
<th>Grade 3/4 AEs, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dabrafenib + Trametinib</strong></td>
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<tr>
<td>COMBI-d(^1) BRAF V600E or V600K–mutated, previously untreated advanced melanoma Primary endpoint: OS</td>
<td>1:1 randomization to:</td>
<td>423</td>
<td>Dabrafenib + trametinib: 69%</td>
<td>Dabrafenib + Trametinib: 11 mo (8.0–13.9)</td>
<td>Dabrafenib + Placebo: 25.1 mo</td>
<td>All: Dab + Tram: 48 Dab + placebo: 50 Pyrexia: Dab + Tram: 59 Dab + placebo: 25 Fatigue: Dab + Tram: 29 Dab + placebo: 37 Nausea: Dab + Tram: 36 Dab + placebo: 27 Cutaneous SCC: Dab + Tram: 4 Dab + placebo: 12</td>
</tr>
<tr>
<td></td>
<td>• Dabrafenib 150 mg twice daily + Trametinib 2 mg once daily</td>
<td></td>
<td>Dabrafenib + placebo: 53%</td>
<td>Dabrafenib + Placebo: 18.7 mo</td>
<td>HR 0.56 (95% CI 0.46–0.69)</td>
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<tr>
<td></td>
<td>• Dabrafenib + placebo</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>COMBI-v(^1) BRAF V600E or V600K–mutated, previously untreated advanced melanoma Primary endpoint: OS</td>
<td>1:1 randomization to:</td>
<td>704</td>
<td>Dabrafenib + trametinib: 64%</td>
<td>Dabrafenib + Trametinib: 11.4 mo</td>
<td>Dabrafenib + Placebo: 25.6 mo</td>
<td>All: Dab + Tram: 52 Vemurafenib: 63 Pyrexia: Dab + Tram: 53 Vemurafenib: 21 Rash: Dab + Tram: 22 Vemurafenib: 43 Nausea: Dab + Tram: 35 Vemurafenib: 36% Arthralgia: Dab + Tram: 24 Vemurafenib: 51</td>
</tr>
<tr>
<td></td>
<td>• Dabrafenib 150 mg twice daily + trametinib 2 mg once daily</td>
<td></td>
<td>Vemurafenib: 51%</td>
<td>Vemurafenib: 18 mo</td>
<td>HR 0.61 (95% CI 0.52–0.73)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Vemurafenib 960 mg twice daily</td>
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</tbody>
</table>

| Encorafenib + Binimetinib                  |                  |     |              |               |              |                 |
|                                           | • Encorafenib 450 mg/day + binimetinib 45 mg twice daily | | Encorafenib: 51% | Encorafenib: 18 | HR 0.54 (95% CI 0.41–0.71) |
|                                           | • Encorafenib 300 mg daily | | Vemurafenib: 40% | Vemurafenib: 10 | |
|                                           | • Vemurafenib 960 mg twice daily | | | |

AE = adverse events; CI = confidence interval; GI = gastrointestinal; HR = hazard ratio; ICC = investigator’s choice chemotherapy; mPFS = median progression-free survival; mOS = median overall survival; ORR = objective response rate.
BRAF and MEK inhibition produced greater responses and improved progression-free and overall survival (Table 2). Interestingly, the rate of cutaneous squamous-cell carcinomas was much lower with combination therapy, reflecting the more profound degree of MAPK pathway inhibition achieved with combination BRAF and MEK inhibition. Based on these results, FDA approval was granted for both dabrafenib/trametinib and vemurafenib/cobimetinib combinations in 2015. Although the dabrafenib/trametinib combination was only approved in 2015, trametinib had independently gained FDA approval in 2013 for the treatment of BRAF V600E/K–mutated melanoma on the basis of the phase 3 METRIC study.77

Encorafenib (LGX818) and binimetinib (MEK162, ARY-162, ARY-438162) are new BRAF and MEK inhibitors currently being evaluated in clinical trials. Encorafenib/binimetinib combination was first evaluated in a phase 3 study (COLUMBUS) that compared it with vemurafenib monotherapy in BRAF-mutant melanoma.78 Unsurprisingly, encorafenib/binimetinib combination produced greater and more durable responses compared to vemurafenib monotherapy. The median progression-free survival of the encorafenib/binimetinib combination (14.9 months) was greater than vemurafenib monotherapy (7.3 months) in this study, and intriguingly greater than that seen with vemurafenib/cobimetinib (coBRIM 9.9 months) and dabrafenib/trametinib (COMBI-d 9.3 months; COMBI-v 11.4 months). Of note, although encorafenib has an IC50 midway between dabrafenib and vemurafenib in cell-free assays (0.8 nM dabrafenib, 4 nM encorafenib, and 31 nM vemurafenib), it has an extremely slower off-rate from BRAF V600E, which results in significantly greater target inhibition in cells following drug wash-out.79 This may account for the significantly greater clinical benefit seen with encorafenib/binimetinib in clinical trials. Final study data are eagerly awaited. Regulatory approval has been sought, and is pending at this time.

Binimetinib has been compared to dacarbazine in a phase 3 study (NEMO) of patients with NRAS-mutant melanoma, most of whom had been previously treated with immunotherapy.80 Response rates were low in both arms, although slightly greater with binimetinib than dacarbazine (15% versus 9%), commensurate with a modest improvement in progression-free survival. FDA approval has been sought and remains pending at this time.

**KIT INHIBITION SINGLY AND IN COMBINATION**

The KIT receptor protein tyrosine kinase is a transmembrane protein consisting of extracellular and intracellular domains. Activating KIT mutations occur in 2% to 8% of all melanoma patients and may be found in all melanoma subtypes but are commonest in acral melanomas (10%–20%) and mucosal melanomas (15%–20%). Activating KIT mutations primarily occur in exons 11 and 13, which code for the juxtamembrane and kinase domains, respectively.5,81–83

Imatinib mesylate is a tyrosine kinase inhibitor of the 2-phenyl amino pyrimidine class that occupies the tyrosine kinase active site with resultant blocking of tyrosine kinase activity. Imatinib mesylate is known to block KIT and has been extensively studied in patients with gastrointestinal stromal tumors (GIST), 80% of whom harbor KIT mutations, in both the adjuvant and the metastatic settings. In melanoma, imatinib mesylate was studied in a Chinese open-label, phase 2 study of imatinib mesylate monotherapy in metastatic melanoma harboring KIT mutation or amplification; 25% of the study patients had mucosal disease and the rest had cutaneous disease, with acral involvement in 50% of all patients.84 Overall response rate was 23%, while 51% of patients remained alive at 1 year with no differences in response rate and/or survival being noted between patients with either KIT mutations or amplifications. In a separate study of imatinib mesylate at 400 mg daily or 400 mg twice daily in
Caucasian patients with KIT-mutated/amplified melanoma, similar response and survival rates were reported, although patients with KIT mutations did nonsignificantly better than those with KIT amplifications.

Other novel studies evaluating KIT inhibitors include KIT inhibition in combination with the VEGF inhibitor bevacizumab and a study of selective BCR-ABL kinase inhibitor nilotinib in imatinib-resistant melanoma. In the former phase 1/2 study, Flaherty and colleagues studied imatinib 800 mg daily and bevacizumab at 10 mg/kg every 2 weeks in 63 patients with advanced tumors, including 23 with metastatic melanoma. Although the combination was relatively nontoxic, no significant efficacy signal was seen and further accrual to the phase 2 portion was halted after the first stage was completed.

Nilotinib is a BCR-ABL1 tyrosine kinase inhibitor intelligently designed based on the structure of the ABL-imatinib complex that is 10 to 30 times more potent than imatinib in inhibiting BCR-ABL1 tyrosine kinase activity. Nilotinib is approved for the treatment of imatinib-resistant chronic myelogenous leukemia (CML), with reported efficacy in patients with central nervous system (CNS) involvement. Nilotinib has been studied in a single study of KIT-mutated/amplified melanoma that included patients with imatinib-resistance and those with treated CNS disease. Nilotinib appeared to be active in imatinib-resistant melanoma, although no responses were seen in the CNS disease cohort. Overall, the response rates observed with KIT inhibition in melanoma are much lower than those observed in CML and GIST.

CONCLUSION AND FUTURE DIRECTIONS

Prior to 2011, the only approved agents for the treatment of advanced melanoma were dacarbazine and high-dose interleukin-2. Since 2011, drug approvals in melanoma have proceeded at a frenetic pace unmatched in any other disease. The primary events underlying this are advances in our understanding of the gene mutation landscape driving melanoma tumorigenesis, accompanied by insights into the means by which tumors circumvent the induction of effective anti-tumor T-cell responses. These insights have resulted in the development of inhibitors targeting MAPK pathway kinases BRAF, MEK, and NRAS, KIT, and regulatory immune checkpoints (CTLA-4 and PD-1). Although BRAF/MEK inhibition results in profound reductions and even occasional complete responses in patients, these responses are typically short lived, rarely lasting more than 9 to 11 months; the encorafenib/binimetinib combination may improve that duration marginally. However, the signature therapeutic advance in melanoma of the past decade is immunotherapy, particularly the development of inhibitors of CTLA-4 and PD-1 immune checkpoints. With these agents, significant proportions of treated patients remain free of progression off-therapy (ipilimumab 23%; nivolumab 34%; pembrolizumab 35%; ipilimumab/nivolumab 64%), and some patients can be successfully reinduced after delayed progression. Separately, the high response rates observed with the use of KIT inhibitors in CML and GIST have not been observed in KIT mutated/amplified melanoma and development of agents in this space has been limited. The challenges ahead center around identifying predictive biomarkers and circumventing primary or acquired resistance, with the eventual goal of producing durable remissions in the majority of treated patients.

Our improved understanding of the mechanisms of acquired resistance to BRAF/MEK inhibitors suggests that anti-tumor activity may be achieved by targeting multiple pathways, possibly with combination regimens comprising other inhibitors and/or immunotherapy. Preclinical data supports the use of combination strategies targeting both ERK and PI3K/mTOR to circumvent acquired resistance. Ongoing studies are evaluating combinations with biguanides (metformin:
Metastatic Cutaneous Melanoma

NCT02143050 and NCT01638676; phenformin: NCT03026517), HSP90 inhibitors (XL888: NCT02721459; AT13387: NCT02097225), and decitabine (NCT01876641).

One complexity affecting management of resistance in the targeted therapy landscape remains tumor heterogeneity, particularly intratumoral heterogeneity, which may explain the apparent contradiction between continued efficacy of BRAF inhibitors in BRAF-resistant tumors and preclinical data predicting slower progression of resistant tumors on cessation of BRAF inhibitors.91–94 These data provide a rationale to investigate intermittent dosing regimens with BRAF/MEK inhibitors; several studies exploring this approach are ongoing (NCT01894672 and NCT02583516).

Given the specificity, adaptability, and memory response associated with immunotherapy, it is likely that these agents will be used to treat the majority of patients regardless of mutational status. Hence, identifying predictive biomarkers of response to immune checkpoint inhibitors is vital. The presence of CD8+ T-cell infiltrate and IFN-γ gene signature, which indicate an “inflamed” tumor microenvironment, are highly predictive of clinical benefit from PD-1 inhibitors.95,96 However, not all PD-1 responders have “inflamed” tumor microenvironments, and not all patients with an “inflamed” tumor microenvironment respond to immune checkpoint inhibitors. The complexity of the immune system is reflected in the multiple non-redundant immunologic pathways, both positive and negative, with checkpoints and ligands that emerge dynamically in response to treatment. Given the dynamic nature of the immune response, it is unlikely that any single immunologic biomarker identified pre-treatment will be completely predictive. Rather, the complexity of the biomarker approach must match the complexity of the immune response elicited, and will likely incorporate multifarious elements including CD8+ T-cell infiltrate, IFN-γ gene signature, and additional elements including microbiome, genetic polymorphisms, and tumor mutation load. The goal is to use multiple markers to guide development of combinations and then, depending on initial response, to examine tumors for alterations to guide decisions about additional treatment(s) to improve responses, with the eventual goal being durable clinical responses for all patients.

**REFERENCES**


