New Strategies in Melanoma: Entering the Era of Combinatorial Therapy

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Abstract

The treatment of metastatic melanoma has been revolutionized over the past decade as effective molecularly targeted therapies and immunotherapies entered the clinic. It is hoped that deeper insights into the characteristics of patients and tumors that are most responsive will allow more precise patient selection for these therapies while understanding mechanisms of resistance will facilitate the development of rational combinations or next-generation agents aimed at novel targets.

Disclosure of Potential Conflicts of Interest

R.J. Sullivan is a consultant/advisory board member for Astex Pharmaceuticals. K.T. Flaherty is a consultant/advisory board member for GlaxoSmithKline, Novartis, and Roche. No other potential conflicts of interest were disclosed.

Editor’s Disclosures

The following editor(s) reported relevant financial relationships: J.L. Abbruzzese is a consultant/advisory board member for Celgene and Halozyme.

CME Staff Planners’ Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should have an improved understanding of the standard treatment options for advanced (metastatic and unresectable) melanoma and the rationale for building “regimens” such as dual BRAF/MEK inhibitor therapy, combined immunotherapy (e.g., ipilimumab plus nivolumab), and the combination of molecular and immune-targeted therapy.

Acknowledgment of Financial or Other Support

This activity does not receive commercial support.

Background

Over the past 5 years, major advances have been made to the standard of care for patients with metastatic melanoma. Over this time interval, the FDA approved six therapies (ipilimumab, vemurafenib, dabrafenib, trametinib, pembrolizumab, and nivolumab) based on the results of remarkable clinical trial data, made even more remarkable given that only two therapies (dacarbazine and high-dose IL2) were approved in the previous 35 years (1–10). With this success have come a few challenges, such as optimal treatment sequencing and combination therapy. In patients with BRAF mutations, retrospective data support a strategy of first-line immunotherapy followed by BRAF-targeted therapy, if needed, and standard BRAF-targeted therapy should be combined BRAF–MEK inhibitor therapy on the heels of three randomized phase III trials showing superiority of combination therapy compared with single-agent BRAF inhibitor therapy (11–16). Finally, the initial data for combined immunotherapy are very encouraging, particularly with the regimen of concurrent ipilimumab and nivolumab (16).

From the mid-1970s through the mid-2000s, an era that saw the rise and approval of dozens of chemotherapeutic agents and the development of curative regimens for several malignancies, the survival of patients with metastatic melanoma went unchanged (17). Also, the discovery of immune cytokines produced only two agents with demonstrated ability to alter the natural history of cancer when given as single agents (18, 19). Only two drugs, the chemotherapy dacarbazine and the cytokine interleukin-2 (IL2), gained regulatory approval by the FDA. During this era, investigators followed the trend in oncology to explore combinations of cytotoxic agents and regimens combining cytotoxics with cytokines, which were unique in the melanoma field. Despite a number of regimens showing promising phase II data, none translated into an overall survival advantage in phase III trials compared with single-agent chemotherapy, the Dartmouth regimen (cisplatin, dacarbazine, vinblastine, and tamoxifen) and biochemotherapy [many versions, most commonly used was cisplatin, dacarbazine, vinblastine, IL2, and interferon-alpha (IFNα)] being two well-
studied examples (20–26). Still, while a number of chemotherapeutic and biologic modifier’s were being tested in melanoma, a number of seminal discoveries were made that have revolutionized therapy for melanoma patients. These findings have directly led to the development of highly effective immune-targeted and signal transduction targeted therapies, and the FDA has approved six agents (ipilimumab, vemurafenib, dabrafenib, trametinib, pembrolizumab, and nivolumab) with four different mechanisms of action (CTLA-4 inhibitor, BRAF inhibitors, MEK inhibitor, and PD-1 receptor inhibitor) since 2011 (1–6, 8). The impact on patients has been profound, as patients are living longer and better. But the effect on the melanoma research and treatment community has been similarly profound, as conversations about how to limit the burden of disease-related symptoms from melanoma have turned to plans of how to cure it.

As with any revolution, the seeds of change were sewn years in advance of this wave of approved agents. Yet, as we take the next steps to improve upon the efficacy of both single-agent immune-targeted and signal transduction targeted therapy, it is only close interaction and collaboration between clinical and bench researchers that will help to uncover the mechanisms of action and resistance to these agents, thereby setting the stage for rational combinatorial regimens. In truth, this is already happening, as combined BRAF and MEK inhibitor regimens have been shown to overcome some mechanisms of resistance to single-agent BRAF inhibition preclinically and translated into improvements in response and survival in randomized clinical trials and the approval of dabrafenib and trametinib in combination for BRAF-mutant melanoma patients (2, 14). With regard to immune-targeted therapy, the combination of ipilimumab and the anti–PD-1 antibody nivolumab has been associated with both very high response rates and toxicity, though clearly it has demonstrated the proof-of-concept that enhanced efficacy is possible with immunotherapy combinations (16). Finally, there have been great efforts to combine BRAF-targeted therapy with immunotherapy, based on compelling data emerging from several groups (27–30). The focus of this review is to describe the advances that have led to the recent approvals in melanoma, present the emerging data from early efforts to build combinatorial regimens, and then look ahead at where the field is moving and predict what the treatment landscape will look like in 5 to 10 years.

**Advances, Limitations, and New Strategies of Immune-Targeted Therapy**

Perhaps the first observation that served as the launching point for immunotherapy in cancer, was that melanoma is immunogenic and under certain circumstances, prone to immune destruction. The first description of effective immunotherapy is likely from Coley, whose ‘toxin’ derived from pathogenic bacteria was injected to cancer patients with the hopes that the resultant systemic inflammatory response would lead to tumor regression (31). In the century that followed Coley’s initial work, the understanding of immune system activation and deactivation clarified. This led to the development of a number of early immune-targeted therapies including the cytokines IFNα and IL2, which both were approved for the treatment of melanoma in the 1990s (18, 32). Still, the most profound immunotherapy-relevant scientific breakthrough was the elucidation of how T lymphocytes are activated and negatively regulated by, so-called, immune checkpoints (Fig. 1; refs. 33, 34). It was from these initial studies that the concept of checkpoint blockade emerged as a viable anticancer strategy and directly led to the clinical development of anti–CTLA-4, anti–PD-1, and anti–PD-L1 antibodies.

**The prototype: anti–CTLA-4 monoclonal antibodies**

The first therapeutic immune checkpoint inhibitors were the anti–CTLA-4 monoclonal antibodies ipilimumab and tremelimumab. The mechanism of action of these agents involves monoclonal antibody engagement of CTLA-4 and antagonization of the CTLA-4 interaction with its binding partners, the antigen-presenting cell (APC) cell-surface markers B7.1 (CD80) and B7.2 (CD86; refs. 35, 36). More recently, it also appears that intratumoral depletion of regulatory T cells is another potential mechanism of action of anti–CTLA-4 treatment (37). Because CTLA-4 is a major negative regulator of T-cell activation, through direct competition for B7.1/B7.2 engagement with the coactivating T-cell surface molecule CD28, inhibition of CTLA-4 is the functional equivalent of ‘taking the brakes off’ of this effector branch of the immune system and allowing for central T-cell activation, which is best characterized by CTLA-4 knockout models where animals die within months after birth from overwhelming lymphoid proliferation and autoimmunity (35). In patients, treatment with anti–CTLA-4 antibodies is indeed associated with significant and occasionally lethal autoimmune toxicity (6, 8, 36). However, ipilimumab was also the first treatment shown to prolong overall survival in patients with metastatic melanoma (6). Specifically, in two randomized, phase III trials, metastatic melanoma patients treated with ipilimumab had a superior overall survival than in patients not randomized to receive ipilimumab, leading to the FDA approval of ipilimumab in 2011. The first newly approved drug for that indication in 15 years (6, 8). Developed over a similar time frame, a second anti–CTLA-4 antibody failed to show superiority in a randomized, phase III trial compared with single-agent chemotherapy, though patients who responded to tremelimumab remained in response substantially longer than those who responded to chemotherapy (35.8 vs. 13.7 months; P = 0.0011; ref. 38). One of the reasons given for the lack of a survival benefit was the fact that a significant number of patients randomized to tremelimumab received ipilimumab either as standard therapy after approval in 2011 or as part of the ipilimumab-expanded access protocol prior to 2011, thereby confounding the interpretation of the primary endpoint (38).

A better mousetrap: anti–PD-1/PD-L1 monoclonal antibodies

Shortly after the identification of CTLA-4 as a major regulator of T-cell activation, a second immune checkpoint, a receptor on T cells named PD-1, was discovered and shown to have an important role in regulating immune activation (39). Soon thereafter, two ligands, PD-L1 and PD-L2, were identified and shown to mediate T-cell inactivation when engaged with PD-1 (33, 40). Importantly, PD-L1 and PD-L2 expression was found not only on APCs but also in tumor and tissues, and the interaction with PD-1 was shown to be a critical mechanism of tissue and tumor self-preservation. More importantly, disruption of PD-1 interaction with tumor-expressed PD-L1 was associated with substantial antitumor immunity in preclinical models (41). It was thus with great anticipation when the first monoclonal anti–PD-1 antibody was tested in human patients with metastatic cancer.

The phase I study of the anti–PD-1 antibody nivolumab, then known as MDX-1106, was a dose-escalation study of single-dose
therapy, followed by a dose expansion cohort that enrolled a total of 39 patients (10 of whom had melanoma; ref. 42). Amazingly, significant tumor regressions were seen in patients with melanoma, colon cancer, renal cell carcinoma, and non–small cell lung cancer, despite the limited drug exposure. Subsequently, a multiple-dose study of nivolumab showed substantial activity in melanoma (28% response rate in 98 patients), as well as other diseases (9). In this original melanoma treatment cohort, the median overall survival was shown to be 16 months and a number of patients remained in remission after discontinuing study treatment after 2 years (10). In the first reported randomized trial of anti–PD-1 antibodies, nivolumab was shown to be superior to chemotherapy in patients with metastatic melanoma who had previously been treated with ipilimumab (43). Specifically, patients treated with nivolumab had a nearly 3-fold increase in response rate (32% vs. 11%) compared with chemotherapy; data for overall and progression-free survival (PFS) was still too immature. Nivolumab was approved by the Japanese regulatory authorities in July 2014, for the treatment of metastatic melanoma, and in December 2014 by the FDA for the treatment of patients with metastatic melanoma after ipilimumab (and BRAF inhibitors in patients with tumors harboring a BRAF mutation). A more recent study comparing first-line nivolumab with dacarbazine in BRAF wild-type, metastatic melanoma demonstrated the clear superiority of anti–PD-1 therapy over chemotherapy with respect to response rate (40% vs. 13.9%; \( P < 0.001 \)), PFS [median, 5.1 vs. 2.2; hazard ratio (HR), 0.43; \( P < 0.001 \)], and overall survival (1 year 72.9% vs. 42.1%; HR for death, 0.42; \( P < 0.001 \); ref. 7). On the basis of these data, it would be reasonable to expect approval in the first-line setting in this patient population in the near future.

A second anti–PD-1 antibody, pembrolizumab (MK-3475, formerly known as lambrolizumab), has also shown remarkable activity both in patients who were and were not treated with prior ipilimumab (4). With confirmed response rates in the 25% to 40% range (depending on whether it was administered before or after ipilimumab), this agent produced target lesion regression in over 70% of the more than 400 patients treated on the phase I trial (4, 44). In addition, median survival is likely to exceed 2 years. As with nivolumab, pembrolizumab is extremely well tolerated and is associated with grade 3 or 4 toxicities in less than 10% of
patients (4, 10). On the basis of its high response and survival rates in patients previously treated with ipilimumab, pembrolizumab received accelerated approval by the FDA in September 2014.

To date, there have now been three anti–PD-L1 antibodies tested extensively in cancer clinical trials, MDX-1105, MPDL3280A, and MEDI4736. The first, MDX-1105 (BMS-936559), was associated with responses in slightly less than 20% of patients with melanoma (9 of 52), with slightly lower response rates in other diseases as well (45). This was very well tolerated, with grade 3/4 toxicity seen in less than 10% of patients. In a phase Ia trial of MPDL3280A, 43 patients with melanoma were enrolled and responses were seen in 28% of cases; all but one responding patient had ongoing responses lasting longer than 6 months with many still ongoing (46). Notably absent are severe autoimmune events, which fits with the hypothesis that disrupting PD-L1–PD-1 interactions, while sparing PD-L2–PD-1 interactions may partially dissect out the antitumor versus antihost effects of targeting this axis (46). More recently, MEDI4736 has been shown to be safe and active in some patients, though data have not been presented or published to date in a cohort of melanoma patients (47).

Vaccines: the next generation

For decades, efforts to develop effective melanoma vaccines were focused on designs that would maximize specific melanocytpeptide antigen expression (GP100, Tyrosinase, MART1, MAGE3). These types of vaccine efforts never yielded single-agent benefit. Taking the GP100 peptide vaccine as an example, this is clearly inferior to ipilimumab as a single agent (6). In combination with ipilimumab, it does not show any enhanced efficacy compared with single-agent ipilimumab (6). Curiously, in combination with high-dose IL2, however, it is associated with an improvement in response rate and survival compared with single-agent IL2 (48). Historical efforts to induce or further promote recognition of lineage-specific or developmental antigens have given way to a more thorough consideration of antigens that may be dispensable to tumors, such as mutated epitopes within oncopgenes (49). With the emergence of immune checkpoint inhibitors in melanoma, the role of vaccines, particularly those not shown to improve efficacy in combination with these agents, has become tenuous. Still, the rationale remains strong that tumor antigen expression is theoretically the critical initiating step in immune recognition and ultimate antitumor immunity, and a new approach to release antigens and recruiting immune elements to those antigens has recently been embarked upon (6, 50). In the absence of an ability to predict which epitopes would be the most immunogenic for an individual patient, a new approach has emerged in which an inflammatory microenvironment is created in situ in one or more of a patient’s metastatic tumors. In this way, antigen-presenting dendritic cells would present a repertoire of antigens that is not informed by the vaccine, but rather in a context that is more favorable to effective immune activation.

Perhaps the exemplar of this next generation of vaccines is talimogene laherparepvec (TVEC), which is an oncolytic herpes virus engineered to express granulocyte macrophage colony-stimulating factor (GM-CSF). In a randomized phase III trial, TVEC injections into palpable subcutaneous tumors were compared with GM-CSF injections in patients with stage III unresectable or stage IV melanoma, and patients randomized to TVEC had a higher rate of “durable response” (51). Although the primary endpoint was a bit controversial, because a traditional response criterion was not used and injected lesions were incorporated into the response assessment, surprisingly the overall survival of patients randomized was better than those randomized to GM-CSF, with a median survival duration of 23.3 months compared with 18.9 months, HR, 0.79, though with borderline P value of 0.051. Furthermore, it appeared that the patients most likely to have an improvement in survival, assessed in a subset analysis, were those with unresectable stage III disease (HR, 0.57; P < 0.001). A second example of next-generation vaccination strategies is IMCgp100, a bifunctional molecule that targets the gp100280–286 Peptide presented by HLA-A2 on one end and recruits T cells via a low-affinity anti-CD3 component on the other end (52). The initial data from the phase I, dose-escalation trial was presented and was associated with definitive tumor responses in at least 2 of the first 10 patients (53).

It’s all about combinations?

Although the data with single-agent immune checkpoint inhibitors are remarkable, particularly with anti–PD-1/PD-L1 therapy, one could make a strong argument that these agents hold their most promise in combination, given that T-cell activation and tumor infiltration represent just one of the key mechanisms of antitumor immunity. Specifically, antigen presentation, antigen recognition, proper cytokine coordination of Th1 response, T-cell costimulation, and T-cell trafficking and tumor infiltration are all required to mount a host response against a tumor (54). Thus, vaccines that efficiently lead to antigen release/presentation in the proper cytokine context (such as TVEC) or that facilitate T-cell trafficking and antigen recognition (IMCgp100) may be a complement to agents that trigger T-cell activation, such as the immune checkpoint inhibitors (51, 53). In addition, Th1 cytokines, such as the type 1 IFNs or IL2, may be an excellent adjuvant to anti–CTLA-4 or anti–PD-1/PD-L1 antibodies (55, 56). Finally, combined immune checkpoint–targeted therapy is generating the most excitement with respect to immunotherapy combinations, as the early combined anti–CTLA-4 plus anti–PD-1 data emerge and as the next wave of monoclonal antibodies to immune checkpoints, such as LAG-3, KIR, and OX40, emerges from single-agent phase I testing and is available for exploration in combination (57–59).

The first combination immunotherapy trial involving checkpoint inhibitors was a phase I/II trial of ipilimumab and high-dose IL2 carried out at the NCI. In all, 36 patients were enrolled and responses were seen in 8 patients (22%). A multicenter study is being launched to evaluate the efficacy of the combination (NCT02203604; ref. 60). A study assessing a second anti–CTLA-4 antibody plus cytokine has also shown interesting results. Specifically, tremelimumab was combined with high-dose IFN in a phase II trial, with response seen in 9 of the 35 evaluable patients (26%), with 4 patients (14%) achieving a complete response (61). Although neither combination of anti–CTLA-4 antibody plus cytokine therapy has become more widely studied, both trials serve as proof-of-concept that this combination is feasible, associated with manageable toxicity profiles, and potentially more effective than single-agent therapy.

The earliest studies of ipilimumab, outside of the phase I trial, were in combination with a variety of peptide vaccines (60, 62–64). None of these strategies was associated with response rates greater than expected with ipilimumab alone, and the registration trial that led to the approval of ipilimumab quite convincingly
showed that ipilimumab alone was as good (or perhaps even slightly better) than ipilimumab in combination with a gp100 vaccine (6). Still, the concept of combined vaccination with anti–CTLA-4 therapy is enticing, and more recently, the combination of TVEC with ipilimumab has shown intriguing early data. In a phase II study, 10 of 18 evaluable patients (56%) had a response to the combination (65). Given the relatively low response rate of both single agents, this dramatically improved response rate appears to represent a synergistic effect of the combination. Importantly, toxicity did not appear to be synergistic and, rather, additive of the expected adverse events of each single agent. Despite the small sample size, this study finally provides proof-of-concept that vaccination may potentiate the effects of immune checkpoint inhibitors, or vice versa. This combination is most definitely deserving of further study in a larger phase II and possibly randomized phase III trials.

Since Bristol-Myers Squibb (BMS) has developed both ipilimumab and nivolumab, it was logical on clinical grounds, particularly with preclinical evidence suggesting potential synergy, for this combination to be investigated for safety and efficacy (57). The phase I trial was performed in patients with metastatic melanoma and produced astounding preliminary data. In the original publication, the data from the first 53 treated patients were reported, and evidence of clinical activity was seen in 65% of patients with 53% of patients having a protocol-defined response, (16). More remarkably, each responder had at least an 80% reduction in measured tumor volume and 2-year survival rates are approximately 70% (66). Not unexpectedly, this combination was associated with substantial immune-related toxicity, and grade 3/4 adverse events were seen in over 50% of patients (16, 66). Whether this combination (or that of ipilimumab plus pembrolizumab: NCT02089685) ultimately proves more effective and is manageable in routine clinical practice compared with sequential single-agent therapy strategies (NCT01783938, NCT01927419, NCT01844505) with anti–PD-1/PD-L1 and anti–CTLA-4 antibodies remains to be seen, though this trial clearly demonstrates the potential of combined targeting of immune checkpoints, and has been quickly followed by a number of similar trials (Table 1).

**Advances, Limitations, and New Strategies of Molecular-Targeted Therapy**

The development of effective molecularly targeted agents for melanoma similarly has been characterized by a number of important fundamental discoveries. The first of these arguably was the discovery of oncogenic NRAS mutations in a subset of melanoma patients in the early to mid-1980s (67). Over the subsequent two decades, signal transduction biology came to the forefront of oncology research with the identification that signaling pathways, such as the P38 and MAPK pathways, are often constitutively activated by either mutated oncogenes or tumor-suppressor genes that drive tumor growth, cell-cycle activation, angiogenesis, inhibition of apoptosis, and immune evasion (68, 69). In 2002, BRAF mutations were described in over half of patients with melanoma, and a new target emerged as the focus of researchers and pharmaceutical companies (70). The subsequent approval of the BRAF inhibitors vemurafenib and dabrafenib, as well as the MEK inhibitor trametinib, validated the biologic importance of this oncogene and the MAPK pathway more generally in melanoma (1, 3, 5).

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<th>Table 1. Immunotherapy combinations with inhibitors of CTLA-4 and PD-1. Green (reported trials), orange (ongoing trials, not yet reported)</th>
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**MAPK pathway/landscape of oncogenic/tumor suppressor gene mutations**

Somatic mutations of oncogenes and tumor-suppressor genes lead to constitutive activation of the MAPK pathway in over 80% to 90% of patients with melanoma (Fig. 2A; ref. 70). With the development of potent inhibitors targeting the mediators of this pathway (Fig. 2B), the treatment of molecularly defined subgroups has improved dramatically in recent years and is expected to improve further over the coming years through rationally designed combination trials based on well-designed preclinical studies and analysis of pretreatment, on-treatment, and time-of-progression tumor biopsies from patients on molecularly targeted therapy.

**Targeting BRAF: from target identification to effective multidrug combinations**

Two factors led to the molecular therapy revolution in melanoma. The first was the identification that BRAF mutations were present in a near majority of melanomas and that these mutations led to constitutive MAPK signaling that drove tumor proliferation and survival (68). The second was the proliferation of small-molecule kinase inhibitors to hit the clinic in the 2000s following the remarkable proof-of-principle findings in chronic myelogenous leukemia that small-molecule target inhibition could lead to dramatic control of malignant disease (71). The earliest tested “RAF inhibitors,” sorafenib and RAF265, were revealed as being multitargeted kinase inhibitors that had no real specificity for RAF, and were challenging to dose at levels high enough for efficient MAPK inhibition due to off-target toxicities related to the multiple other kinases that are inhibited by these agents. Not surprisingly, these agents never were associated with the dramatic effects of the more potent and specific BRAF inhibitors (72–74).

The first of these second-generation BRAF inhibitors to reach the clinic was vemurafenib. In the phase I trial, responses were limited to patients harboring BRAFV600 mutations and were seen in the great majority of such patients (75). A second selective and...
Figure 2. A, oncogenic mutations (red) and tumor-suppressor gene mutation (dark gray) that activate the MAPK pathway. B, inhibitors of the MAPK pathway.
potent BRAF inhibitor, dabrafenib, showed similar outcomes as vemurafenib in early phase I studies (76). On the basis of the early results with both agents, randomized phase III trials of each drug compared with a chemotherapy control arm were performed and showed superiority of BRAF inhibitor therapy with respect to response rates (approximately 50% for BRAF inhibitors vs. 5%–7% for dacarbazine), PFS (HR, 0.26 for vemurafenib, \(P < 0.001\); HR, 0.30 for dabrafenib, \(P < 0.0001\)), and overall survival (HR, 0.37 for vemurafenib, \(P < 0.001\); HR for dabrafenib not available; refs. 1, 5). On the basis of these data, the FDA approved vemurafenib in 2011 and dabrafenib in 2013. A third potent and specific BRAF inhibitor, encorafenib, has also shown promise in phase I/II testing and is currently being evaluated in phase III trials (77).

Allosteric inhibitors of MEK1/2 have been in development for the better part of a decade. In the early phase I trials with various MEK inhibitors, there were often exceptional responses that occurred in patients with metastatic melanoma (78–80). In addition, there were strong preclinical data that BRAF-mutant melanomas would be particularly vulnerable to these agents. To date, two agents, trametinib and binimetinib, have been shown to have significant response rates (~20%) and PFS (4–5 months) in patients with BRAF-mutant melanoma (3, 81). There are also data from a randomized phase III clinical trial of trametinib compared with chemotherapy, showing superiority of the MEK inhibitor with respect to response rate, PFS, and overall survival. These data led to the FDA approval of trametinib in patients with BRAF-mutant melanoma in 2013 (3).

Despite the successes of single-agent BRAF and MEK inhibitors for the treatment of metastatic BRAF-mutant melanoma, the reality is that most patients have developed disease progression by 6 or 7 months and only a small percentage of patients remain progression-free beyond a year (1, 5, 75, 82). Although there is great interest in determining who this small percentage of patients will be, there has been even more effort placed into determining why the majority of patients developed resistant disease. The earliest reports identified that at the time of acquired resistance, there were two dominant subtypes, ERK-dependent, where the MAPK pathway was reactivated, and ERK-independent, where the MAPK pathway remained inhibited by BRAF-targeted therapy (83–86). Other studies suggested that there were also mechanisms of resistance that were “intrinsic” to the tumor, meaning they could be identified prior to commencement of therapy and were associated with poorer outcomes when present, and often involved genetic aberrations (loss of tumor suppressors such as PTEN and/or CDKN2A), genomic amplifications (BCL2A1, CCND1, CDK4), or growth signals from the tumor microenvironment (hepatocyte growth factor; refs. 87–90). The multitude of identified mechanisms of resistance, devising combinatorial regimens has been a challenging proposition, though one combination strategy was embarked upon early in the development of each drug and changed the way BRAF-mutant melanoma was treated, just 2.5 years after the approval of vemurafenib.

On the basis of the finding that a majority of patients with acquired resistance to BRAF inhibitors had upregulation of the MAPK pathway after initial inhibition, a strategy was developed to look at the combination of BRAF and MEK inhibitors for the treatment of BRAF-mutant melanoma (2, 83). One logical concern regarding this concept is that maximal inhibition of the MAPK pathway, while perhaps leading to enhanced tumor killing, would be intolerable. Interestingly, this concern was unfounded, as BRAF inhibitors actually led to paradoxical activation in BRAF wild-type cells that are driven to signal through the MAPK pathway (91, 92). The best-described clinical example of this is the fact that BRAF inhibitors are associated with the development of cutaneous squamous cell carcinomas (cuSCC) or keratoacanthomas (KA) in over 20% of patients (1, 5, 76, 83). In one series, HRAS mutations were seen in a majority of these cuSCC/KAs (93).

As a proof-of-concept, the phase I trial of dabrafenib and trametinib determined that the maximally tolerated dose (MTD) of the combination was at the individual MTDs of single-agent dabrafenib (150 mg twice daily) and trametinib (2 mg daily; ref. 2). Furthermore, the rate of cuSCC, KAs, and other cutaneous toxicity was substantially decreased in combination than when compared with single-agent BRAF inhibitors. More importantly, the efficacy of combined dabrafenib and trametinib at MTD was shown to be better than single-agent dabrafenib in the phase II randomized study, with regard to response rate (76% vs. 54%) and PFS (HR, 0.39; \(P < 0.001\)). Perhaps more importantly, an update of this trial, presented at the ASCO 2014 meeting, showed that the median survival for patients in the MTD combination group was 23.8 months and the 12-month survival rate was 80% (94). Moreover, a significant subset of patients continued to receive combined BRAF and MEK inhibitors after disease progression (94). This dataset led to the FDA approval of the combination for the treatment of advanced, BRAF-mutant melanoma.

These data have now been corroborated in two randomized phase III trials comparing the combination of dabrafenib and trametinib to single-agent dabrafenib (COMBI-d) and vemurafenib (COMBI-v). Following the initial analysis of COMBI-d for PFS and overall survival, it was determined that the trial met its primary endpoint of PFS reduction (HR, 0.75; \(P = 0.035\)) and overall survival benefit as well (HR, 0.63; \(P = 0.023\)), both favoring combination therapy (14, 95). Similarly, the COMBI-v trial was stopped after a preplanned preliminary analysis because the combination arm was associated with improved overall survival (HR, 0.69; \(P = 0.005\)) and PFS (HR, 0.56; \(P < 0.001\); median, 11.4 vs. 7.3 months) compared with single-agent vemurafenib (15). In both of these phase III trials, the toxicity rates were similar in both groups, with the combination being associated with a higher rate of febrile syndrome and a lower rate of cutaneous toxicity, including cuSCC/KA (14, 15).

On the basis of the original clinical findings with the combination of dabrafenib and trametinib, it was expected that the other two potent and specific BRAF inhibitors would be tested in combination with MEK inhibitors as well. Indeed, the combination of vemurafenib and the MEK inhibitor cobimetinib and the combination of encorafenib and binimetinib have been evaluated and shown to be associated with excellent clinical outcomes as well as remarkable toxicity profiles (96, 97). The more developed of these two combinations in that of vemurafenib and cobimetinib. Again, it was noted that the MTD, determined in the dose-escalation study, was the combination of the agents at the individual agent’s MTD, and that responses occurred in over 80% of patients (97). In a randomized, phase III trial of the combination compared with single-agent vemurafenib showed that the combination was associated with improved PFS (HR, 0.51; \(P < 0.001\); median, 9.9 vs. 6.2 months) and 1-year survival, but was too early in analysis to show a statistically significant benefit in overall survival (13). The third combination, encorafenib plus binimetinib currently is in phase III testing (NCI 01909453), though interestingly the MTD of the combination involves a dose of encorafenib.
that involve four or inhibitor. Moving forward, either with triplet regimens or those addition of a BCL2/BCL-x antagonist, HSP90 inhibitors, HDM2 strategies in the clinic are summarized in Table 1, and include the third drug to the BRAF plus MEK inhibitor backbone. The current under way to develop triplet combinations that involve adding a mechanisms of this resistance are very similar to that of single-agent BRAF inhibitor therapy and most often involve reactivation of the MAPK (98). Thus, over the past 1 to 2 years, a sea change is under way to develop triplet combinations that involve adding a third drug to the BRAF plus MEK inhibitor backbone. The current strategies in the clinic are summarized in Table 1, and include the addition of a BCL2/BCL-x antagonist, HSP90 inhibitors, HDM2 inhibitor, a MET inhibitor, a PI3K inhibitor, and a CDK4/6 inhibitor. Moving forward, either with triplet regimens or those that involve four or five agents, it is expected that continuous dosing of all agents will not be feasible due to toxicity. As such, it will be incumbent upon the research community to vet alternative sequencing and combinations to maximize efficacy while limiting toxicity.

New strategies for an old enemy: effectively targeting NRAS

As previously described, mutations in NRAS were the first oncogenic mutations identified in melanoma, nearly 20 years preceding the discovery of BRAF mutations. Over the past several years, it has been solidified that activating NRAS mutations are found in at least 20% of melanomas, signal through the MAPK pathway, and a significant subset are susceptible to MEK inhibition, at least preclinically (70, 99). The first substantial clinical evidence of MEK inhibitor activity in NRAS-mutant melanoma was from a trial of the MEK inhibitor binimetinib. In this phase II study, binimetinib was associated with a 20% response rate and a median PFS between 4 and 5 months (81). This finding has led to a randomized, phase III trial of binimetinib versus chemotherapy in patients with NRAS-mutant melanoma (NCT01763164). Although the initial data are encouraging, the bottom line is that even if the phase III trial of binimetinib demonstrates a significant improvement in PFS, its primary endpoint, the great majority of patients will be in need of next-line therapy within 6 to 8 months (81). Combination therapy may be warranted, but much less is known about resistance to MEK inhibitors in NRAS-mutant melanoma than resistance to BRAF inhibitors in BRAF-mutant melanoma, at a similar time point in the development of these agents in the clinic.

Fortunately, this is changing. The first emerging molecularly targeted, combinatorial strategy for NRAS-mutant melanoma is based on an elegant, unbiased experiment in which the investigators screened the gene expression of preclinical animal models of NRAS-mutant melanoma in three different conditions: (i) vehicle control; (ii) MEK inhibitor; and (iii) NRAS extinction (100). When comparing the differences in gene expression, it became clear that genes regulating the cell cycle, particularly cyclin-dependent kinase 4 (CDK4), were overexpressed in the MEK inhibitor–treated tumors compared with the NRAS extinction tumors. The authors then showed that the combination of a MEK inhibitor and a CDK4/6 inhibitor was associated with better outcomes than either single agent alone. The first clinical trial of a MEK-plus-CDK4/6 inhibitor (binimetinib plus LEE011) combination demonstrated tolerability at reasonable doses of both agents, reduction in tumor volume in most patients, and a 33% response rate in NRAS-mutant patients (101). The second such trial is a phase I trial of trametinib and palbociclib and is currently in dose escalation (NCT02065063).

The convergence of immune and molecular targeting

Following the approval of ipilimumab and vemurafenib in 2011, there was significant pressure from the two companies that licensed these agents, BMS and Roche Genentech, respectively, to open a clinical trial combining these agents. Although the impetus for this was likely political in response to the lobbying efforts of patient advocate groups, there was emerging evidence that suggested a scientific rationale for this trial. Specifically, a number of groups showed that BRAF mutations suppressed melanocytic antigen expression and that this was relieved in the setting of BRAF inhibitors, thereby leading to enhanced antigen expression (27, 102). Furthermore, analysis of tumors obtained prior to and on treatment with BRAF inhibitors showed that not only did melanocytic antigen expression increase, but so too did the number of immune cells, including, and likely most importantly, CD8-positive T cells (28, 30, 103). The most straightforward conclusion that can be drawn from these data are that BRAF inhibition is associated with changes in the immune-microenvironment that could be postulated to enhance the effectiveness of immunotherapy. Two additional pieces of data are that BRAF inhibitors do not exert immunosuppressive effects on T cells, based on in vivo studies, and that the combination of BRAF and MEK inhibitors appears to be associated with similar effects on the immune microenvironment and similarly is not associated with immunosuppressive effects on T cells (27, 28).

The first clinical trial of combined immunotherapy and molecularly targeted therapy was indeed the phase I trial of vemurafenib and ipilimumab (29). In this dose-escalation study that involved a 28-day lead-in phase of vemurafenib followed by concurrent therapy, 12 patients were enrolled to two cohorts, yet only 10 received combination therapy. This is because 6 of the first 10 patients to be treated with the combination had grade 3 or 4 elevation of transaminase levels. The remaining 2 patients in the vemurafenib lead-in phase did not receive the combination due to the closure of this trial based on the determination that the drugs could not be safely combined. The results of this trial certainly affirm the concept that even approved drugs should not be combined outside of carefully controlled clinical studies. Still, these findings have not deterred the interest in combined immune and molecular targeting strategies. Currently, there are multiple trials evaluating a number of combination approaches in BRAF-mutant and wild-type populations that are summarized in Table 2.

Conclusions

It is staggering to imagine that 4 years ago there were two approved drugs for melanoma, dacarbazine and high-dose IL2, and no proven combination strategies for the treatment of metastatic melanoma. In a metaphorical blink of an eye, we now have multiple immune and molecular targeted therapies approved, amazing data with combinations of molecularly targeted approaches in BRAF- and NRAS-mutant melanoma populations and combinations of immune-targeted approaches for all patients with melanoma, and emerging rationales for many additional combinatorial strategies. There is no doubt that the future treatment of melanoma will involve therapeutic regimens that include multiple agents, given together and in sequence, with wide varieties of molecularly defined and immunologic targets.
remain that need to be solved. These include the following:

1. Determination of the optimal sequencing of molecular and immune-targeted therapy
2. Identification of effective targeted therapy regimens for BRAFV600 wild-type subtypes
3. Optimization of combination immune-targeted therapy regimens
4. Development of BRAF-targeted therapies using triplet (or even quadruplet) regimens and alternative dosing schedules
5. Evaluation of approved agents for metastatic/unresectable melanoma in the high-risk stage II and III settings

To address these issues, carefully designed clinical and translational research studies will need to be performed across multiple institutions and investigators. In addition, a focus on predictive blood and tissue-based biomarker development will be essential to truly understand which patients should be receiving a particular therapy/regimen and why.

**References**

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