

Combined targeted therapy and immunotherapy in melanoma: a review of the impact on the tumor microenvironment and outcomes of early clinical trials

Meredith S. Pelster and Rodabe N. Amaria

Abstract: The development of BRAF and MEK inhibitors (BRAFi and MEKi) and immune checkpoint inhibitors have changed the management of advanced stage melanoma and improved the outcomes of patients with this malignancy. However, both therapeutic approaches have limitations, including a limited duration of benefit in subsets of BRAF-mutant melanoma patients treated with targeted therapy and a lower overall response rate without a clear predictive biomarker in patients treated with checkpoint inhibitors. Preclinical and translational data have shown that BRAFi and MEKi alter the tumor microenvironment to make it more amenable to immunotherapy and have provided the scientific rationale for combining BRAFi and MEKi with immunotherapy. In this review, the initial studies demonstrating the impact of BRAFi and MEKi on the expression of melanoma differentiation antigens, T-cell infiltration, and the balance of immune stimulatory and immune suppressive cells and cytokines are addressed. Preclinical work on the combination of targeted therapy with BRAFi and MEKi with immunotherapy are reviewed, highlighting improved tumor responses in mouse models of BRAF-mutated melanoma treated with combinatorial strategies. Lastly, data from early clinical trials of combined targeted therapy and immunotherapy are discussed, focusing on response rates and toxicities.

Keywords: BRAF inhibitor, immunotherapy, MEK inhibitor, melanoma, targeted therapy, tumor microenvironment

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Introduction

The management of advanced stage melanoma has dramatically changed with the development of molecularly targeted therapies and immunotherapies, both of which improve the overall survival (OS) of patients with metastatic disease.^{1–8} Since 2011, three BRAF inhibitor (BRAFi) and MEK inhibitor (MEKi) combinations, which inhibit the mitogen-activated protein (MAP) kinase pathway, have been approved for the treatment of BRAF-mutated melanoma.^{2,4,5} The immune checkpoint inhibitors ipilimumab [cytotoxic T-lymphocyte-associated protein (CTLA)-4 blockade], nivolumab and pembrolizumab [programmed cell death (PD)-1 blockade] have also

been approved for the treatment of advanced melanoma regardless of mutation status.^{3,6,7} Overall, these new agents have improved the outcomes of advanced melanoma and have been able to effectively cure a subset of patients.

Despite the recent progress with these agents, both therapeutic approaches have limitations. While BRAFi and MEKi have a high overall response rate (ORR) in patients with BRAF-mutated melanoma, their effect can be short-lived with the majority of patients developing resistance to these drugs and subsequent progressive disease. Immunotherapy agents can lead to durable responses for some patients, but the ORR is

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Correspondence to:
Rodabe N. Amaria
The University of Texas MD
Anderson Cancer Center,
Department of Melanoma
Medical Oncology, 1515
Holcombe Boulevard, Unit
0430, Houston, TX 77030,
USA
rnamaria@mdanderson.org

Meredith S. Pelster
The University of Texas
MD Anderson Cancer
Center, Department of
Hematology/Oncology,
Houston, TX, USA



lower, and there is not a clear biomarker indicating which patients are more likely to benefit.

There has been interest in combining targeted therapy and immunotherapy in patients with advanced disease due to the complementary strengths and weaknesses of these two therapeutic approaches. Additionally, preclinical and clinical data have shown that BRAFi and MEKi affect the tumor microenvironment and tumor immunogenicity in many ways, providing further support for the investigation of combinations with immunotherapy. Emerging preclinical and clinical data show that combining BRAFi and MEKi with immunotherapy can be beneficial, albeit with many unanswered questions regarding the choice of drugs, the best sequence or timing of initiating the therapeutic agents, and how to best mitigate toxicity.

In this review we will address the initial studies that demonstrated the effects of BRAFi and MEKi on the tumor microenvironment and anti-tumor immunity as well as data supporting the benefit of combination targeted and immunotherapy in metastatic melanoma.

Effect on the tumor microenvironment and anti-tumor immunity by BRAF and MEK inhibition: preclinical data

Laboratory analysis of BRAF-mutated melanoma has demonstrated immunologically ‘cold’ features, with low T-cell infiltrates and increased immunosuppressive cells, regulatory T-cells (Tregs), myeloid-derived suppressor cells (MDSCs), and cytokines such as interleukin (IL)-6, and IL-10.^{9–11} In addition, the low expression of melanoma differentiation antigens (MDAs) and down-regulation of major histocompatibility complex (MHC) expression in BRAF-mutated melanoma decreases tumor recognition by the immune system.¹²

These unfavorable immunologic features are reversed by BRAFi and MEKi therapy. Preclinical studies using both melanoma cell lines and mouse models of BRAF-mutant melanoma have demonstrated that treatment with BRAFi monotherapy increases T-cell infiltration into tumors, upregulates MDA expression including MART-1, gp-100, TYRP-1, and TYRP-2, and enhances MHC I and II expression *via* an increase in interferon-gamma.^{13–15} MEKi therapy similarly has been shown to upregulate MDA expression in both BRAF-mutant and BRAF wild-type melanoma.¹³ T-cells have demonstrated increased

activity in these models, with an increase in interferon-gamma release, enhanced CD40L expression, and improved cytotoxicity.^{10,13,14} Importantly, the use of BRAKi or MEKi therapy has also been shown to increase the number of MDA-specific T-cells.^{13,16} MEKi therapy in particular has been demonstrated to decrease effector CD8+ T-cell death *via* chronic antigen stimulation.¹⁶ Other preclinical studies have shown a decrease in Tregs and MDSCs in mouse models of melanoma treated with BRAFi-targeted therapy and an increase in the activity of antigen-presenting dendritic cells.^{17–19}

Effect on the tumor microenvironment and anti-tumor immunity by BRAF and MEK inhibition: translational data

Translational studies of the effects of BRAFi and MEKi therapy on patient tumor samples have similarly demonstrated a favorable impact on the tumor microenvironment and immunogenicity. Multiple longitudinal studies of patients with BRAF-mutated melanoma treated with BRAFi monotherapy or BRAFi and MEKi combination therapy show an increase in CD4 and CD8 T-cell infiltration in patient tumor samples taken while patients are early on therapy. Wilmott and colleagues examined pretreatment biopsies and biopsies after 7 days of treatment with either dabrafenib or vemurafenib and showed an increase in T-cell infiltration that correlated with a reduction in tumor size and increased degree of tumor necrosis.²⁰ Similarly, a study of patients treated with dabrafenib or dabrafenib and trametinib documented a significant increase in CD8 T-cell infiltration on day 10–14 biopsies compared with pretreatment biopsies.⁹ Kavakand and colleagues documented similar results, and Cooper and colleagues additionally showed an increase in the clonality of the tumor-infiltrating lymphocytes in on-treatment biopsy specimens, suggesting that the T-cells are proliferating in response to antigens present in the tumor.^{21,22}

In addition to demonstrating an increase in T-cell infiltration, Frederick and colleagues showed increased T-cell cytotoxicity with increased levels of granzyme B and perforin after 10–14 days of dabrafenib or combination dabrafenib and trametinib.⁹ The same group also importantly showed an induction of MDA expression after targeted therapy treatment, documenting an approximately 5–14 fold increase in expression of MART-1, gp-100, TYRP-1, and TYRP-2.⁹ This

evidence provides further support of the favorable immunological effects of BRAFi and MEKi on the melanoma tumor microenvironment.⁹

Additional studies have demonstrated an effect of targeted therapy on the levels of immune stimulatory and suppressive molecules, cytokines, and cells. The serum of patients treated with BRAFi monotherapy or BRAFi and MEKi combination therapy was found to have higher levels of the immune stimulatory cytokines interferon-gamma, tumor necrosis factor (TNF)- α , and CCL4 and lower levels of the immune suppressive cytokine IL-8.²³ A decrease in IL-8 as well as another immune suppressive cytokine, IL-6, was also noted in melanoma biopsy specimens of patients on targeted therapy for 10–14 days.⁹ Liu and colleagues additionally showed a decrease in vascular endothelial growth factor production in patient tumor samples after treatment with targeted therapy, documenting another method by which these agents alter the tumor environment favorably.¹⁴ Lastly, evaluation of the serum of patients treated with vemurafenib has shown decreased levels of immunosuppressive MDSCs, mirroring preclinical findings.²⁴

In summary, data from both preclinical models and patient tissue and blood samples show that targeted therapy with BRAFi or a combination of BRAFi and MEKi alter the tumor microenvironment and immunogenicity in melanoma through a variety of mechanisms, including increased infiltration and activity of T-cells, enhanced MDA expression and presentation, and a favorable change in immune stimulatory and suppressive cytokine levels.

Immune mechanisms of resistance to BRAF and MEK inhibitor therapy

Clinical trials and the experience of subsequent patient care show that major limitations of targeted therapy include the often limited duration of effectiveness and the aggressive nature of the melanoma once resistance to BRAFi and MEKi is seen.²⁵ Research demonstrating the beneficial effects of targeted therapy with BRAFi and MEKi on anti-tumor immunity has simultaneously shown that immune mechanisms also contribute to the development of resistance to these agents.

Patient biopsies taken at the time of progression on targeted therapy show both a decrease in

T-cell infiltration and MDA expression.^{9,26} Additionally, T-cells that are present in the tumor microenvironment show markers of immune exhaustion such as expression of PD-1 and TIM3.⁹ Interestingly, these T-cell markers are also found in on-treatment biopsy specimens prior to progression.⁹ Pieper and colleagues further demonstrated in *in vitro* studies that long-term treatment of melanoma cells with BRAFi or a combination of BRAFi and MEKi led to a decrease in CD8 tumor-infiltrating lymphocyte activity.²⁷ They went on to show that this decrease in T-cell activity is mediated by down-regulation of MDA expression, which occurs by 2 weeks of targeted therapy exposure.²⁷ Other researchers working with melanoma cell lines have additionally noted that these cells express higher levels of the immune inhibitory molecule programmed death ligand (PD-L)1 after exposure to BRAFi.²⁸

To summarize, while BRAFi and MEKi therapy has been shown to have initial favorable effects on the tumor microenvironment and immunogenicity, a decrease in T-cell infiltration and activity, a decrease in MDA expression, and an increase in inhibitory molecule expression develops in patients who progress. This development of immune-mediated resistance to targeted therapy has provided a scientific rationale to support the investigation of targeted therapy and immunotherapy combinations.

Combination targeted therapy and immunotherapy effects on anti-tumor immunity

Preclinical data strongly support the synergy between targeted therapy and immunotherapy in the treatment of melanoma *via* improving anti-tumor immunity. In the SM1 mouse model of BRAF-V600E-driven melanoma, Hu-Lieskovan and colleagues showed that the combination of dabrafenib, trametinib, and a mouse anti-PD-1 antibody led to improved tumor responses compared with either targeted therapy or immunotherapy alone.²⁹

Also, using the SM1 mouse model, Moreno and colleagues demonstrated an improved anti-tumor response with a combination of dabrafenib, trametinib, and an anti-PD-1 antibody.³⁰ They went on to test additional immune-stimulating antibodies against CD137 and CD134 and showed that the addition of one of these antibodies to make

a four-drug regimen was superior to the three-drug regimen of dabrafenib, trametinib, and anti-PD-1 antibody.³⁰

Cooper and colleagues developed a novel BRAF-V600E/Pten^{-/-} syngeneic tumor graft immunocompetent mouse model of melanoma and tested BRAFi with immunotherapy agents.³¹ They found a 7.5-fold increase in T-cell tumor infiltration when a BRAFi was combined with either an anti-PD-1 or an anti-PD-L1 antibody compared with monotherapy with either of these agents.³¹ They also noted a higher CD8:Treg ratio, suggesting a more favorable tumor microenvironment, as well as enhanced T-cell activity with increased granzyme B, interferon-gamma, and TNF- α production.³¹ In this same study, Cooper and colleagues reported results of an analysis of longitudinal biopsy specimens of a patient with metastatic melanoma who was treated sequentially with 4 weeks of BRAFi therapy and four courses of an anti-CTLA4 antibody. The tissue after 4 weeks of BRAFi therapy showed few tumor-infiltrating lymphocytes, suggesting that some immune-mediated resistance had developed at this time point; however, after a dose of anti-CTLA4 antibody, the T-cell infiltrate increased and persisted.³¹ Further analysis showed a favorable CD8:Treg ratio after anti-CTLA4 antibody treatment.³¹

Using a similar mouse model of BRAF-driven melanoma, Deken and colleagues also tested the combination of BRAFi and MEKi therapy with anti-PD-1 immunotherapy.³² Based on prior data suggesting a time-limited beneficial immune effect of targeted therapy, the mice were treated with 14 days of targeted therapy agents with or without a continuously dosed anti-PD-1 antibody.³² Tumor volume reduction with the combination of BRAFis and MEKis and anti-PD-1 was significantly improved compared with targeted therapy alone; an increase in the proportion of animals achieving a complete response was also noted, with some animals having durable responses of up to 200 days. This beneficial effect was shown to be mediated through CD8 T-cells.³²

In summary, preclinical studies of the combination of targeted therapy and immunotherapy in BRAF-mutated melanoma mouse models show a further beneficial effect on the tumor microenvironment and improved tumor responses, with the potential of durable complete responses.

Clinical outcomes of combination targeted therapy and checkpoint inhibitor immunotherapy

In addition to preclinical data on combinatorial strategies, retrospective clinical data of patients who have been treated with both targeted therapy and immunotherapy have been analyzed and provide insights. A 2014 study of a cohort of patients treated with targeted therapy, including BRAFis alone or in combination with MEKis, assessed treatment responses when targeted therapy was given before or after immunotherapy, which included anti-CTLA4 agents, anti-PD-1 agents, and IL-2.³³ A total of 32 patients had received targeted therapy after immunotherapy and had an ORR of 57% with a progression-free survival (PFS) of 5.6 months and an OS of 19.6 months, indicating that patients had an acceptable response to targeted therapy subsequent to immunotherapy.³³ Of 242 patients who initially received targeted therapy, 40 progressed and went on to receive the anti-CTLA4 antibody ipilimumab; in this situation response rates were poor with no complete or partial responses observed and only two patients with stable disease.³³ PFS was 2.7 months, and OS was 5.0 months for this cohort.³³ In another retrospective analysis of patients who had received targeted therapy prior to pembrolizumab, similar results were found with a poor disease control rate of 18.6% and a PFS of 3.0 months.³⁴ These studies provide preliminary evidence suggesting that the use of immunotherapy after targeted therapy progression may be insufficient to provide durable response. However, it must be noted that these data are not from randomized trials and should be interpreted cautiously.

Other experience outside of clinical trials provides insights regarding combinatorial therapy. Investigators from Germany reported a case series of 10 patients with metastatic BRAF-mutated melanoma, including 6 patients with brain metastases, who were treated sequentially with targeted therapy and immunotherapy.³⁵ These patients were first treated with vemurafenib for a median of 11.5 weeks followed by the addition of ipilimumab for 4 cycles.³⁵ Overall, 2 patients developed grade 4 transaminitis which resolved with steroid therapy.³⁵ A response assessment after 12 weeks of ipilimumab revealed 5 patients with a partial response, 2 patients with stable disease, and 3 patients with progressive disease.³⁵ In 5 of the 7 patients with a partial response or stable disease, vemurafenib was stopped with repeat

assessment after 2 months; 3 of these 5 patients had no further progression of disease for at least 1 year.³⁵ Overall the median PFS was 8.0 months, and the OS was 13.0 months.³⁵

With mounting preclinical evidence of the synergistic effects of targeted therapy and immunotherapy and retrospective and case series data available to guide drug timing, researchers have designed prospective clinical trials to assess the efficacy of combinations (Table 1).

An initial phase I trial assessed the combination of vemurafenib and ipilimumab in patients with metastatic BRAF-mutated melanoma; vemurafenib was given as a single agent for 1 month initially followed by 3 mg/kg ipilimumab every 3 weeks with continued concurrent vemurafenib.⁴⁴ Unfortunately the trial was terminated due to the high frequency of hepatotoxicity with the combination: 6 of the 10 patients who received the combination developed grade 3 transaminitis, with most requiring glucocorticoids for treatment.⁴⁴ This early study demonstrates that the choice of targeted and immunotherapy agents and their side-effect profiles is critical when considering combination therapy.

Another early trial tested the combinations of dabrafenib and ipilimumab or dabrafenib, trametinib, and ipilimumab in metastatic melanoma patients and similarly found significant toxicity in the triplet arm.⁴⁵ Patients on the triplet therapy regimen were treated with a run-in period of 14 days of targeted therapy with dabrafenib and trametinib followed by ipilimumab 3 mg/kg.⁴⁵ Overall, 2 of the 7 patients that received this regimen developed colitis with intestinal perforation, and this arm of the study was closed.⁴⁵ Minor and colleagues note that on the dabrafenib with ipilimumab arm, 1 of 25 patients experienced colitis without perforation, raising the question of whether the interaction of trametinib with ipilimumab was the cause of increased risk of colitis.⁴⁵

Amin and colleagues assessed sequential targeted therapy and immunotherapy in a single-arm, open-label phase II study of 6 weeks of vemurafenib followed by ipilimumab in previously untreated patients with advanced BRAF-mutated melanoma.⁴¹ A total of 46 patients were treated.⁴¹ Grade 3 or 4 drug-related adverse effects were noted in 65.2% of patients, with no drug-related deaths.⁴¹ Of note, 21.7% of patients experienced

grade 3 or 4 gastrointestinal toxicity, and 4.3% experienced grade 3 or 4 hepatobiliary toxicity.⁴¹ Exploratory outcome endpoints were reported, including complete response in 4.3%, partial response in 28.3%, stable disease in 10.9%, and progressive disease in 23.9%.⁴¹ The median duration of response to sequential therapy was 23.1 months.⁴¹ The median PFS was 4.5 months, and the median OS was 18.5 months.⁴¹ Patients who progressed after ipilimumab were permitted to resume vemurafenib, with an overall response to re-treatment of 36.8%.⁴¹

More recent, ongoing trials of combination therapy have reported fewer toxicities and encouraging initial results. In a phase Ib dose-escalation study of vemurafenib, cobimetinib, and the anti-PD-L1 immunotherapy agent atezolizumab in BRAF-mutant melanoma patients, an unconfirmed response rate of 85.3% in the first 34 patients was noted.³⁶ Grade 3 or 4 therapy-related adverse events were reported in 44.1% of patients.³⁶ A total of 3 patients discontinued therapy due to transaminitis, and one discontinued due to a rash.³⁶ The phase I/II KEYNOTE-022 trial is investigating the combination of dabrafenib, trametinib, and pembrolizumab in previously untreated patients with advanced BRAF-mutated melanoma.^{38,47} Initial results of the phase I study show a 67% unconfirmed ORR, with the median duration of response not met.³⁸ A total of 20% of patients had dose-limiting toxicities (neutropenia and transaminitis), while overall 73% experienced grade 3 or 4 treatment-related toxicities.³⁸ It was noted that all immune-mediated adverse effects, including pneumonitis, rash, anterior uveitis, hepatitis, hyperthyroidism, and hypothyroidism, resolved.³⁸ Results from the randomized phase II component of KEYNOTE-022 were recently reported.³⁹ Patients with BRAF-mutated advanced melanoma were randomized to receive concurrent dabrafenib and trametinib with pembrolizumab or placebo, with 60 patients in each arm.³⁹ The response rate was interestingly lower in the pembrolizumab group than in the placebo group (63% versus 72%).³⁹ Additionally, the trial did not meet its prespecified endpoint of improved median PFS, though there was a clear trend in the direction of longer PFS with pembrolizumab (the median PFS with pembrolizumab was 16.0 months compared with 10.3 months with placebo).³⁹ It is notable that more patients receiving pembrolizumab had responses lasting longer than 18 months than those receiving placebo

Table 1. Summary of recent and ongoing targeted therapy and checkpoint inhibitor immunotherapy combination clinical trials.

Trial	Phase	Therapy	Status	Preliminary outcomes	Notable toxicities
Trials with preliminary outcome data available					
NCT01656642 ³⁶	Ib	V + C 1-month run-in, V + C + Atezo	Enrollment complete	ORR 85.3%	44.1% of patients with grade 3–4 treatment-related toxicities
NCT01988896 ³⁷	Ib	C + Atezo in BRAF-WT and BRAF-mutant	Enrollment complete	ORR 45.0%	54.5% of patients with grade 3–4 treatment-related toxicities
NCT02130466 ^{38,39} KEYNOTE-022	I/II	BRAF-mutant: D + T + pembro BRAF-WT concurrent: T 1-month run-in, T + pembro BRAF-WT intermittent: T intermittent + pembro	Recruiting	Phase I: ORR 67% in BRAF-mutant phase II (D + T + pembro <i>versus</i> D + T + placebo) in BRAF-mutant: ORR 63% <i>versus</i> 72%	73% of patients with grade 3–4 treatment-related toxicities
NCT02027961 ⁴⁰	I	BRAF-mutant: D + T + durva BRAF-WT concurrent: T + durva BRAF-WT sequential: T → durva	Enrollment complete	ORR 76% in BRAF-mutant ORR 21% BRAF-WT concurrent ORR 50% BRAF-WT sequential	39% of patients with grade 3–4 treatment-related toxicities in BRAF-mutant, 40% in BRAF-WT concurrent, 17% in BRAF-WT sequential
NCT01673854 ⁴¹	II	V → Ipi Resume V if progression after Ipi	Enrollment complete	BOR rate 32.6%	65.2% of patients with grade 3–4 treatment-related toxicities
NCT02910700 ⁴² TRIDeNT	II	D + T + nivo	Recruiting	ORR 91%	21% discontinued study due to toxicity (hepatitis, nephritis)
NCT02967692 ⁴³ COMBI-I	III, part 1	D + T + PDR001 (spartalizumab)	Part 1 completed, part 3 recruiting	ORR 100%	22% discontinued PDR001 due to toxicity (hepatitis, transaminitis)
Trials without preliminary outcome data					
NCT01400451 ⁴⁴	I	V 1-month run-in, V + Ipi	Terminated	Not reported	6/10 patients with grade 3 transaminitis
NCT01767454 ⁴⁵	I	Doublet arm: D 2-week run-in, D + Ipi Triplet arm: D + T 2-week run-in, D + T + Ipi	Completed Triplet arm terminated early	Not reported	2/7 patients on triplet arm developed colitis with intestinal perforation
NCT02908672 ⁴⁶ TRILOGY IMspire 150	III	Placebo: V + C Experimental: V + C 1-month run-in, V + C + atezo	Enrollment complete	Not reported	Not reported
atezo, atezolizumab; BOR, best overall response; D, dabrafenib; durva, durvalumab; Ipi, ipilimumab; NCT, National Clinical Trials identifier; nivo, nivolumab; ORR, overall response rate; pembro, pembrolizumab; T, trametinib; V, vemurafenib.					

(60% versus 28%).³⁹ Grade 3 or higher toxicities occurred in 58% of the pembrolizumab group compared with 27% of the placebo group.³⁹ Of note, in subsequent parts of the KEYNOTE-022 trial, trametinib will be tested with pembrolizumab in patients with BRAF-wild-type melanoma and other solid tumors, both with simultaneous administration of the drugs and with intermittent administration of trametinib. Patients on the intermittent trametinib arm will be on therapy for 2 weeks and off therapy for 1 week of each 3-week cycle; pembrolizumab will be given on day 1 of each cycle.⁴⁸

Another ongoing phase I trial is also assessing combination therapy in patients with both BRAF-mutated and BRAF-wild-type advanced melanoma.⁴⁰ BRAF-mutated patients are treated with dabrafenib, trametinib, and durvalumab, an anti-PD-L1 antibody, while BRAF-wild-type patients are treated either with trametinib and durvalumab or sequential trametinib followed by durvalumab.⁴⁰ Initial results from the first 50 patients treated show a response rate of 76% (16/21) in BRAF-mutated patients treated with the triple combination, a response rate of 21% (3/14) in BRAF-wild-type patients on combination therapy, and a response rate of 50% (3/6) in BRAF-wild-type patients on sequential therapy.⁴⁰ Overall, 2 patients experienced dose-limiting toxicities of thrombocytopenia and choroidal effusion.⁴⁰ In the wild-type cohorts, fewer patients in the sequential therapy group experienced grade 3 or 4 treatment-related toxicities (17%) than in the combination group (40%).⁴⁰ A total of 39% of patients in the BRAF-mutant triple therapy group experienced grade 3 or 4 treatment-related toxicities.⁴⁰

In a tumor-specific expansion cohort of another phase Ib trial, metastatic melanoma patients, including BRAF-mutant and BRAF-wild-type patients, received cobimetinib concurrently with atezolizumab. A response rate of 45.0% was noted, reportedly with similar response rates for BRAF-mutant and BRAF-wild-type patients. Grade 3 or 4 adverse events occurred in 54.5% of patients. The most common toxicities were diarrhea and skin rash.³⁷

A phase II study of dabrafenib, trametinib, and nivolumab in BRAF-mutated advanced melanoma patients has also shown promising early results.⁴² The 3 drugs in this trial are administered concurrently without a targeted therapy

run-in period.⁴² A unique feature of the study design is the permission of patients with untreated, asymptomatic or mildly symptomatic brain metastases to enroll.⁴² A total of 14 patients had enrolled at the time of reporting, with 11 assessed for response.⁴² The ORR reported is 91% with 10 of the 11 patients achieving a partial response.⁴² Overall, 3 patients discontinued the study due to drug toxicity, including immune-mediated hepatitis and nephritis.⁴²

The phase III COMBI-I study is evaluating dabrafenib, trametinib and PDR001, an anti-PD-1 antibody now known as spartalizumab, in patients with advanced BRAF-mutated melanoma.^{43,49} Results for the initial nine patients treated in the part 1 safety run-in component of the trial show that all nine patients responded with 33% complete responses and 67% partial responses.⁴³ Grade 3 or 4 adverse events, reported in more than 1 patient, included hepatitis, increased lipase, and increased transaminases.⁴³ Preliminary results from the part 2 biomarker cohort, also show signals for efficacy and safety.⁴⁹ All 7 patients evaluable after 12 weeks of therapy had unconfirmed partial responses, and the only grade 3 or 4 adverse event to occur in more than 1 patient was pyrexia.⁴⁹ The part 3, randomized controlled trial of the triplet therapy regimen versus dabrafenib, trametinib, and placebo is currently enrolling.

The phase III TRILOGY IMspire 150 trial is also assessing triplet combinatorial therapy, in this case comparing vemurafenib, cobimetinib, and atezolizumab with vemurafenib and cobimetinib alone in previously untreated BRAF-mutated metastatic melanoma patients.⁴⁶ This trial design includes a run-in period of 28 days of targeted therapy prior to the initiation of atezolizumab. Results from the 500 patients treated on this trial are eagerly awaited.

In summary, initial trials of combination targeted therapy and immunotherapy revealed the potential for increased toxicity, including hepatitis and colitis which was more prevalent in trials using vemurafenib and ipilimumab. However, subsequent early-phase trials with use of anti-PD-1 antibodies have indicated acceptable rates of toxicity as well as promising initial response rates. Trials of both concurrent combination therapy as well as sequential combination therapy are ongoing, with much anticipated results regarding confirmed response rates and duration of response.

Clinical outcomes of combination targeted therapy and other immunotherapies

In addition to checkpoint inhibitors, investigators have studied the combination of targeted therapy with other forms of immunotherapy, such as cytokines and adoptive cell therapy.

Clark and colleagues examined sequential treatment with vemurafenib and high dose (HD) IL-2 in a phase II trial. Included patients had BRAF-mutated metastatic melanoma and were enrolled into 2 cohorts.⁵⁰ Patients in the first cohort were previously untreated and received an initial 6 weeks of vemurafenib; patients in the second cohort received vemurafenib for 7 to 18 weeks prior to enrollment.⁵⁰ Both cohorts received HD IL-2 after vemurafenib treatment and were assessed for response.⁵⁰ The complete response rate for the combined cohorts was 27% with a 3-year OS of 30% for the first cohort and 27% for the second cohort.⁵⁰ There were no unexpected toxicities reported.⁵⁰ Overall, the study was not felt to show a synergistic benefit of vemurafenib and HD IL-2 as the results were similar to that for either drug alone.

In another phase II trial, Mooradian and colleagues examined concurrent treatment with vemurafenib and HD IL-2 in 6 patients with advanced BRAF-mutated melanoma.⁵¹ Specifically, patients received vemurafenib for 2 weeks and then began HD IL-2 cycles, during which time they remained on daily vemurafenib.⁵¹ The therapy was well tolerated without unexpected toxicities.⁵¹ The ORR was 83.3%; however, all patients eventually progressed, with a median PFS of 35.8 weeks.⁵¹ Correlative studies of longitudinal tumor biopsies indicated an increase in Tregs in the tumor microenvironment related to HD IL-2 therapy, and the authors hypothesize that this finding may explain the poor duration of response seen with the combination of vemurafenib and HD IL-2.⁵¹

Lastly, Deniger and colleagues conducted a small pilot trial of vemurafenib combined with adoptive cell therapy in metastatic melanoma patients.⁵² In this study, patients underwent tumor resection for growth of tumor-infiltrated lymphocytes followed by 2 weeks of vemurafenib.⁵² Subsequently, they received conditioning chemotherapy, tumor-infiltrated lymphocyte infusion, and high dose IL-2 with the concurrent resumption of vemurafenib, which was continued for up to 2 years.⁵² A total of 64% of the 11 treated patients had an objective response, including 2 patients with a

lasting complete response for 3 years.⁵² The therapy was overall, well tolerated.⁵²

In summary, studies of sequential or combined targeted therapy with HD IL-2 have not shown strong evidence of synergy, while adoptive cell therapy combined with targeted therapy has demonstrated the potential for durable responses in an early study. Further research in this area is ongoing.

Conclusion

There is strong evidence that treatment with BRAFis and MEKis in BRAF-mutated melanoma augments tumor immunogenicity through multiple mechanisms. However, resistance to these targeted therapies frequently develops, which has been shown to be driven in part by immune-mediated mechanisms in addition to the development of molecular resistance. Preclinical work on the combination of targeted therapy with immunotherapy has demonstrated both a positive effect on the tumor microenvironment, including an increase in T-cell infiltration of the tumor (including the demonstration of MDA-specific and clonal tumor-infiltrating T-cells), increased T-cell activity, and a decrease in tumor-suppressive Tregs and MDSCs, and a potential therapeutic benefit in mouse models. Building on this foundation, combination regimens have been tested in melanoma patients in clinical trials, with initial results of combinatorial strategies of BRAFis and MEKis with immune checkpoint inhibitors suggestive of both an increased response as well as additional toxicities. Further results of larger trials are eagerly anticipated to better characterize responses, including duration of response and response to subsequent therapies in patients who progress, as well as toxicities.

A key question remains regarding the optimal timing of combination therapy; some trials begin targeted therapy and immunotherapy simultaneously, while others include a run-in period of targeted therapy of up to a few weeks. The preclinical data suggest that targeted therapy improves the tumor microenvironment by 7–14 days; however, features of immune-mediated resistance may develop soon thereafter.

While most studies have focused on BRAFis and MEKis with immunotherapy for BRAF-mutant melanoma, emerging data show the potential benefit of MEKis in combination with immunotherapy in BRAF-wild-type melanoma.

The phase I trial of durvalumab with dabrafenib and trametinib and KEYNOTE-022 are designed to include both BRAF-mutated and BRAF-wild-type disease. BRAF-wild-type patients will be treated with trametinib and immunotherapy (durvalumab or pembrolizumab). The durvalumab trial will test the combination both concurrent and sequentially, while KEYNOTE-022 will test trametinib given concurrently with pembrolizumab and trametinib dosed intermittently during pembrolizumab therapy.

The results of this as well as multiple other ongoing trials, including the large, randomized phase III TRILOGY IMspire 150 trial, will help to answer questions regarding the safety and efficacy of targeted therapy and immunotherapy combinations in advanced melanoma patients.

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