BRAF inhibitor (BRAFi) treatment enhances antitumor immunity, but is associated with increased intratumoral PD-L1 expression. MEK inhibitors (MEKi) may alter T-cell function; however, recent studies demonstrate preserved T-cell infiltrate during treatment with BRAFi/MEKi. These data have important implications for combining BRAFi/MEKi and checkpoint blockade in the treatment of melanoma. Clin Cancer Res; 21(14) July 15, 2015. ©2015 AACR. See related article by Kakavand et al., p. 3140

In this issue of Clinical Cancer Research, Kakavand and colleagues (1) report that treatment of melanoma patients with combined BRAF inhibitors (BRAFi) and MEK inhibitors (MEKi) is associated with an increase in intratumoral lymphocytes (CD4+ and CD8+), which is no different than that observed when patients are treated with BRAFi monotherapy. The results were observed on analysis of longitudinal tumor samples from 40 patients treated with either BRAFi or combined BRAFi/MEKi, in an effort to better understand the intratumoral immune effects of these agents. In addition to their observations regarding preserved T-cell infiltrate in BRAFi versus BRAFi/MEKi, the group also reported that high PD-L1 expression in patients was associated with increased tumor-infiltrating lymphocytes (TIL) in pretreatment biopsies. Furthermore, these patients had the largest increase in TIL and PD-1 on treatment compared with those with low PD-L1 expression in the pretreatment sample. Of note, no significant difference was found in survival in these groups, although the sample size was relatively small. Overall, these results are important and provide an additional rationale for combining immune checkpoint blockade with BRAFi/MEKi to enhance responses to melanoma therapy, and also speak to the importance of timing and sequence of therapy.

Over the past several years, there have been significant advances in melanoma treatment through the use of targeted therapy and immune checkpoint blockade; however, each of these strategies has limitations as monotherapy. Treatment with BRAFi monotherapy or combined BRAFi/MEKi results in a survival benefit in patients with melanoma, which led to the FDA approval of these regimens (2); however, responses are not durable in the majority of patients. Treatment with immune checkpoint blockade (such as anti–CTLA-4 and anti–PD-1) also results in improved survival, which led to the FDA approval of these agents (3, 4), although a significant proportion of patients do not benefit from this type of therapy.

There is a strong clinical rationale for combining targeted therapy and immune checkpoint blockade in the treatment of melanoma, and a growing scientific rationale supports such combinations. The first scientific data supporting this notion were published in 2010, and demonstrated that blocking oncogenic BRAF activity through targeted MAP kinase pathway inhibition in vitro led to increased melanoma antigen expression and enhanced reactivity to antigen-specific T lymphocytes (5). Importantly, this effect not only was observed with BRAFi in melanomas harboring a BRAFV600E mutation, but was also observed in BRAF wild-type cell lines upon treatment with a MEKi. However, treatment of T cells in vitro with a MEKi resulted in impaired T-cell function, whereas treatment with a BRAFi had no effect on T-cell function. Several groups then studied immune effects of BRAFi ± MEKi in patients with melanoma on therapy, demonstrating enhanced T-cell infiltrate (6), as well as a more favorable tumor microenvironment overall within 2 weeks of treatment initiation—with a decrease in immunosuppressive cytokines and VEGF (6, 7). However, there was a concurrent increase in expression of PD-L1 early on-treatment, suggesting a possible immune mechanism of resistance (6). Interestingly, BRAFi may even stimulate T-cell function through paradoxical signaling via the RAS–RAF pathway (8).

Early clinical studies combining immunotherapy with targeted therapy have largely used BRAFi as a backbone for combinations given the potential for MEKi to alter T-cell function in vitro (5). However, more recently, MEKi have been added to BRAF-targeted therapy in combination with immune-based strategies, and there is growing evidence that it may not "MEK" a difference (9). This has been studied in vitro, and groups have shown that treatment of BRAF wild-type cell lines with MEKi is associated with enhanced melanoma antigen expression (5, 9) and apoptosis in tumor cell lines with increased expression of HLA I and/or HLA II (9). Importantly, investigators have reported a partial but transient inhibition of T-cell proliferation and function upon MEK inhibition (9), which likely relates to T-cell activation status at the time of treatment. Furthermore, synergy is demonstrated...
when combining the MEKi trametinib with immune checkpoint blockade (anti–PD-1, anti–PD-L1, and anti–CTLA-4) in murine models. The findings in patients reported by Kakavand and colleagues are supportive of this notion and suggest little to no deleterious effect of MEK inhibition in combination with BRAF-targeted therapy in patients with melanoma (1). Together, these findings have important potential clinical implications in the care of patients with melanoma and with nonmelanoma malignancies. In patients with melanoma harboring a BRAFV600E mutation, the addition of MEKi to a backbone of BRAF-targeted therapy does not appear to significantly alter T-cell infiltrate (although function was not completely evaluated by Kakavand and colleagues, ref. 1). In patients with BRAF wild-type melanoma, it may be possible to treat concurrently with a MEKi and immune checkpoint blockade, although this concept must be tested in the context of preclinical studies and clinical trials. Similarly, MEKi or other targeted agents may potentially be used in combination with immune checkpoint blockade in the treatment of nonmelanoma malignancies (Fig. 1). This concept is not novel, as preclinical data suggest that treatment with a c-kit inhibitor in gastrointestinal stromal tumors (GIST) enhances T-cell infiltrate in a murine model (10). In this model, treatment of mice with GIST using combined imatinib and anti–CTLA-4 demonstrated synergy with delayed tumor outgrowth and

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Figure 1.
Immune effects of targeted therapy and the potential of adding immune checkpoint blockade. Treatment with a BRAFi results in favorable effects, such as an increase in antigen expression and CD8+ T-cell infiltrate and a decrease in immunosuppressive cytokines and VEGF. However, concurrently, there is an increase in expression of immunomodulatory molecules (PD-1 and PD-L1). Importantly, this therapy requires a BRAFV600E mutation, and the antitumor effect is limited. Treatment with MEKi monotherapy is not as well studied as there are no published data on immune effects of MEKi on the tumor microenvironment in melanoma patients; however, in vitro studies suggest a transient altered phenotype in T cells after MEKi monotherapy. In MEKi monotherapy, there is no requirement for a BRAF mutation, and MEKi can be used in non-BRAF (e.g., RAS)-mutant tumors. Treatment with combined BRAFi + MEKi has the same favorable effects of BRAFi, with similar changes in PD-1 and PD-L1 expression. The addition of immune checkpoint blockade to a backbone of BRAFi and/or MEKi is hypothesized to enhance immune response and overall response to therapy via recruitment and activation of TIL (anti–CTLA-4) and through blocking of PD-1 or its ligand.
prolonged survival. This concept is now being tested in clinical trials.

Despite the enthusiasm for combining these approaches, several caveats exist. First, appropriate timing and sequence are unknown, although recent studies would suggest that the immune response to targeted therapy is early and transient (11) and that these therapies should be used concurrently. This observation is supported by data in the article by Kakavand and colleagues, which suggest a ‘window of opportunity’ for the addition of immune checkpoint blockade onto a backbone of combined BRAF/MEK inhibition (1). Second, unexpected toxicities have been observed in some of the clinical trials combining targeted therapy and immune checkpoint blockade (12); thus, potential toxicity of these combinations must be taken into consideration, and ideally patients should be treated with these strategies in the context of close monitoring on a clinical trial. Third, potential synergy (and related toxicity) of targeted therapy and immune checkpoint blockade in BRAF wild-type melanoma and nonmelanoma malignancies is also unclear; thus, such treatment strategies should be used in the context of close monitoring on clinical trials. Finally, it is critical for us as clinicians and scientists to optimally understand responses to therapy through longitudinal blood and tumor sampling from patients on therapy (as suggested by Kakavand and colleagues), as well as through the use of translational studies in murine models. Ultimately, ideal combination strategies will be built on a deep understanding of the molecular and immune effects of each of these strategies in isolation, as well as in combination.

Disclosure of Potential Conflicts of Interest

J.A. Wargo reports receiving speakers bureau honoraria from DAVA Oncology, and is a consultant/advisory board member for GlaxoSmithKline and Roche/Genentech. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: Z.A. Cooper, J.A. Wargo

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.A. Wargo

Writing, review, and/or revision of the manuscript: Z.A. Cooper, A. Reuben, J. Austin-Breneman, J.A. Wargo

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.A. Wargo

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# Does It MEK a Difference? Understanding Immune Effects of Targeted Therapy

Zachary A. Cooper, Alexandre Reuben, Jacob Austin-Breneman, et al.


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