Raising the bar: optimizing combinations of targeted therapy and immunotherapy

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Major breakthroughs have arisen in the treatment of melanoma and other cancers through the use of targeted and immunotherapy. Therapies targeting the $BRAF^{V600E}$ mutation, such as vemurafenib and dabrafenib, were FDA-approved in 2011 and 2013, following demonstration of rapid, marked response in a majority of patients expressing the $BRAF^{V600}$ mutation and a survival benefit over then standard-of-care therapy with dacarbazine (1,2). However, the vast majority of responding patients eventually relapse, most often within only 6-12 months of treatment initiation (3,4). Another form of immunotherapy, immune checkpoint blockade, exploits a tumor-deployed immune escape mechanism through which tumors impede the immune response by binding checkpoint molecules which serve as brakes, specifically on T lymphocytes. Such therapies involving monoclonal blocking antibodies against cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) were approved in 2011 and 2014, respectively. Though these treatments are associated with responses in fewer patients (20-35%) (5,6) than treatment with targeted therapy, responses are often durable (7) with a significant proportion of patients achieving durable disease control. Unfortunately, many patients do not derive benefit from these forms of therapy (1,2,5,6), and more therapeutic options are needed. Another form of therapy that has been studied extensively is adoptive cell therapy (ACT), and involves the isolation and expansion of antigen-specific lymphocytes from tumor (tumor infiltrating lymphocytes-TIL) (8) or peripheral blood (9) from patients with melanoma (and other cancer types). This form of therapy is associated with responses in approximately 50% of metastatic melanoma patients (10), though its use has been limited by the technical expertise involved in isolation and expansion of these cells, as well as the infrastructure required for this therapeutic approach (11).

Given the success and limitations of each of these forms of therapy, there has been great interest in exploring combination strategies incorporating the use of targeted therapy and immunotherapy with several clinical trials incorporating combination approaches currently underway (NCT01940809, NCT01767454, NCT02200562). However complexities exist, as the effect of targeted therapies on host immune cells is not completely understood, and there is evidence that certain targeted agents (e.g., MEK inhibitors) may have deleterious effects on T cells *in vitro*. Pre-clinical studies deeply examining the mechanism of such approaches are growing in the literature, and will ultimately help inform further combination strategies in patients with melanoma and other cancers. An excellent example of such a paper was recently published in *Science Translational Medicine* (12). In their paper entitled "Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in $BRAF^{V600E}$ melanoma", Hu-Lieskovan *et al*. demonstrated superior antitumor effect when dual targeted therapies with BRAF and MEK inhibitors were combined with immunotherapeutic approaches (including ACT and immune checkpoint blockade with anti-PD-1) in a murine model of $BRAF^{V600E}$ mutant metastatic melanoma.

Studies such as these are critically important, as

targeted therapy agents (such as those targeting $BRAF^{V600E}$ and MEK) succeed in inhibiting crucial signaling and proliferation pathways activated in cancers, but also have unintended effects on the tumor microenvironment, including on immune infiltrates (13). These effects can be advantageous, but at times may also be deleterious. Treatment with BRAF inhibitor therapy is associated with early and significant increases in $CD8^+$ TIL numbers and clonality within tumors of patients with melanoma (14,15), as well as increases in their cytotoxic potential through production of granzyme B and perforin (14). Importantly, these effects may in part be mediated through increases in the expression of human leukocyte antigens (HLAs) (16) and melanoma differentiation antigens (MDAs) gp100, TRP1, TRP2, and MART-1, due to increased transcription of MITF targets as a consequence of MAPK pathway inhibition (14). These favorable changes in the tumor microenvironment are found early during the course of therapy (within the first few weeks) and may be lost later during the course of treatment or at progression on BRAF inhibitors, which has important implications for timing of therapy. Interestingly, BRAF inhibitors manifest paradoxical effects on distinct cell types: they impede proliferation of $BRAF^{V600}$ cells through MAPK pathway inhibition, while causing MAPK pathway hyperactivation in $BRAF^{WT}$ cells such as T lymphocytes, both *in vitro* and *in vivo* (17,18).

There was a strong clinical rationale early in testing to empirically combine BRAF-targeted therapy with immunotherapy, in the hopes of achieving high response rates to therapy (such as those observed in targeted therapy) with a longer duration of response (as seen with immunotherapy). Since then, a growing scientific rationale has suggested combining these forms of therapy. A strong example of this is the observation that although BRAF inhibition is associated with a favorable immune microenvironment early in the course of therapy, there is also an increase in the expression of the immunomodulatory ligand for PD-1 (PD-L1) in the tumor microenvironment, suggesting a potential mechanism of adaptive resistance (14,19). Later studies focused on addressing this through combination of BRAF inhibitors and anti-PD-1 therapy in a murine model, and demonstrated improved anti-tumor responses and survival in combination therapy as opposed to monotherapy with either agent, as well as increased TIL number and activation in tumors of mice on combined targeted therapy and PD-1 blockade (15).

However, immune effects linked to MEK inhibition have been less well characterized, though there is a growing body of observations suggesting that MEK inhibition of T cells is not substantiated *in vivo*. This is crucial, as combining MEK inhibition with immunotherapy could prove significant in $BRAF^{WT}$ melanoma as well as in multiple other cancer types, as many of these are RAS-driven and could be targeted using MEK inhibitors. This is even relevant in BRAF-mutant melanoma, as the current standard of care treatment for patients with metastatic BRAF-mutant melanoma incorporates combined BRAF and MEK inhibition, based on a survival benefit when compared to BRAF inhibitors alone (20).

Multiple *in vitro* studies have been performed to assess effects of MEK inhibitors (21) and suggest that though there are some beneficial effects, MEK inhibitors may impair T cell function. Beneficial effects include enhanced antigen expression in melanoma, as treatment with MEK inhibitors in BRAF wild-type and BRAF mutant melanoma is associated with increased expression of melanoma antigens (such as MART-1, gp100, TRP1 and TRP2) (22), which could be exploited by administering antigen-specific T lymphocytes via ACT.

However, studies have also demonstrated that MEK inhibition may be detrimental to immune cell populations *in vitro* (22,23). Several studies have shown that treatment with MEK inhibitors leads to impaired T cell proliferation, cytokine secretion, and expansion of antigen-specific T cells (22,23). Importantly, suppressive effects of MEK inhibition were not limited to lymphocytes, as it was demonstrated that MEK inhibition also leads to increased maturation of dendritic cells, resulting in decreased cross-presentation and dampened T cell priming (23,24). MEK inhibition may also affect T cell subsets differentially. In the context of graft versus host disease (GVHD) in the setting of stem cell transplants, it was observed that MEK expression is higher in less differentiated and naive T cells, while decreased in effector memory T cells. As shown by Shindo *et al*. (21), MEK inhibition preferentially affected cytokine production and reactivity of naive and central memory T cells, while sparing more differentiated effector memory T cells. This work suggests that MEK inhibition has the potential to detrimentally affect the immune system *in vitro*, but must be further characterized *in vivo* in order to better understand mechanisms of MEK inhibition and the impact of combination strategies with BRAF inhibition and checkpoint blockade immunotherapy.

These issues were elegantly addressed in a manuscript recently published in *Science Translational Medicine* by Hu-Lieskovan *et al*. (12). In these studies, the authors

investigated the synergy of BRAF and MEK inhibition with immunotherapeutic regimens such as ACT and checkpoint blockade immunotherapy. The group studied this in the context of melanoma via a murine model, and used $CD8^+$ T cells from transgenic mice that are specific against the melanoma antigen gp100. In these studies, mice were treated with targeted therapy including dabrafenib (BRAF inhibition) and/or trametinib (MEK inhibition), either as targeted therapy alone or in combination with gp100-specific T cells via ACT. It was demonstrated that treatment with dual targeted therapy through combined dabrafenib and trametinib appears to synergize and offer better tumor control than with monotherapy using either agent alone. These studies were extended to combinations with immunotherapy through ACT, and showed total control of tumor burden.

Importantly, the efficacy of targeted therapy has been linked to the immune response, which could provide a rationale for the success of combinations with ACT. Therefore, T cells were injected into targeted therapy-treated animals and TIL were quantified. Combination of targeted therapy and ACT demonstrated increased homing of T cells to tumors compared to ACT alone within 5 days of treatment, an effect which persisted throughout the course of experiments. Interestingly, the combination of dabrafenib and trametinib presented no improvement in comparison to trametinib alone in enhancing T cell homing to tumors.

One of the previous pitfalls linked to using MEK inhibition has been demonstration of decreased proliferation and viability compared to untreated or BRAF inhibitor-treated T cells *in vitro*. These *in vitro* results were reproduced by Hu-Lieskovan *et al*. However, *in vivo* experiments showed dramatically different findings. Upon injection, it was determined that production of IFN-γ by TIL was unaffected by trametinib treatment. Furthermore, cytotoxicity assays revealed that treatment with dabrafenib and/or trametinib does not impede antigen-specific tumor cytotoxicity, an observation that could be related to differential effects of MEK inhibition on differentiated T cells (12,21).

Another immune escape strategy employed by tumors is their induction of immunosuppressive cell populations such as regulatory T cells (Treg). The impact of combination therapies on this population was therefore assessed, and showed that dabrafenib treatment causes an induction of Tregs in tumors within 5 days following treatment initiation, which could likely have inhibitory effects on the anti-tumor response. Importantly, combination of dabrafenib and trametinib reversed these effects, reducing Treg numbers to their control levels and providing further rationale for combining these targeted therapies. In a set of mechanistic studies, transcriptional analysis by microarray was performed and hierarchical clustering demonstrated that dabrafenib and trametinib showed immune signatures associated to chemokine and MHC expression, as well as PD-L1 up-regulation, which could suggest emergence of a resistance mechanism and rationale for combining targeted therapy with PD-1 blockade.

Accordingly, Hu-Lieskovan and colleagues also investigated the impact of combining targeted therapy with PD-1 blockade. Previous experiments had suggested that, although ACT and targeted therapy may synergize, this leads to both induction of Tregs within tumors, and expression of PD-L1, potentially as a consequence of increased immune reactivity and IFN-γ production within the tumor microenvironment. In subsequent combination therapies including dual dabrafenib and trametinib targeted therapy and PD-1 checkpoint blockade, this triple combination best controlled tumor growth *in vivo*, likely through blockade of PD-1/PD-L1 interaction between tumor and T cells.

Numerous effective treatment regimens have been developed for patients with metastatic melanoma over the past several years, each with its strengths and weaknesses. However, the vast majority of patients do not achieve durable benefit. These pre-clinical studies suggest that combination strategies may be advantageous and overcome the shortcomings of individual monotherapy approaches. Despite this, several other questions remain. First, can combining targeted and immunotherapies benefit patients in the clinical context, and could this extend to other malignancies? Second, considering the rapid and transient immune response induced by targeted therapies, what is the optimal timing and sequence of combination? Third, can combining targeted and immunotherapy increase (or potentially decrease) toxicity? And lastly, can targeting both essential tumor pathways and immune mechanisms circumvent the established tumor heterogeneity? More work is needed to better understand the mechanisms of synergy between these treatments, as well as how translatable these findings may be to melanoma patients in clinical trials, in the hope of optimizing these therapeutic regimens.

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Footnote

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