Therapeutic targeting and monitoring tumor immunity in melanoma

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Background

There have been significant advances in melanoma therapy over the past several years, with several molecularly targeted and immunotherapeutic agents recently FDA-approved for use the treatment of patients with metastatic disease. Notable among these are inhibitors of the BRAF/MAPK pathway (targeted therapy), and antibodies that block immunomodulatory molecules on the surface of T cells and tumors (immune checkpoint inhibitors). However, with these advances, we are posed with therapeutic dilemmas with regard to timing and sequence of therapy. Namely, there is significant debate as to whether to begin treatment with targeted therapy versus immunotherapy upfront, and at which point to change treatment strategy. This is highly relevant, as each of these treatments as mono-therapy have significant limitations.

As a group, we have focused significant effort on better understanding response and resistance to therapy through longitudinal tissue and blood analyses in patients on targeted therapy and immunotherapy. We have worked with investigators worldwide to better understand molecular and immune mechanisms of response and resistance to therapy, and have gained critical insights which have led to therapeutic inroads for patients with melanoma (1, 2). This includes the use of combination strategies, such as adding immune checkpoint blockade to a backbone of molecularly targeted therapy for patients with melanoma (2). Clinical trials combining these strategies are currently underway and response data are not yet mature. However, it is becoming increasingly apparent that complexities exist with regard to these combinations.

A better understanding of mechanisms of response and resistance to combination strategies through translational research is critical, and is best performed on longitudinal patient samples during the course of therapy, which may inform (and be informed by) parallel in vitro and murine studies. Ultimately, ideal approaches combining molecularly targeted therapy and immunotherapy will be built on a deep understanding of the molecular and immune effects of each of these therapies in isolation, as well as in combination.
Discussion

A decade ago, metastatic melanoma was universally fatal, without good treatment options. However, over the past several years, several advances in therapy have been made using insights gained through translational research. Together, these advances have resulted in significantly improved survival rates for patients with metastatic melanoma (3–8).

The first of these advances is in the use of molecularly targeted therapy for melanoma. This is based on the fact that key mutations occur in melanoma tumors that contribute to their oncogenic potential, but can also serve as targets for therapy. A key example of this is the BRAF gene. Mutations in BRAF occur in over half of melanoma tumors, and these activating “oncogenic” mutations result in multiple deleterious effects (9–11). Therapeutic targeting of this oncogenic mutation represents one of the most significant advances in the treatment of melanoma in decades, with up to 80 percent of patients with metastatic melanoma treated with BRAF inhibitors who achieve clinical benefit (4, 7). However, this therapy is limited by a short duration of response, with progression-free survival of less than seven months with BRAF inhibitor monotherapy (4, 7) and less than 10 months with combined BRAF and MEK inhibitor therapy (5). Significant research efforts have focused on better understanding resistance to therapy, and therapeutic strategies incorporating treatment based on identified resistance mechanisms are currently being tested in clinical trials.

Another significant advance in the treatment of melanoma involves the use of immune checkpoint blockade, including the use of monoclonal antibodies targeting immunomodulatory molecules on the surface of T-lymphocytes (or their ligands). These immunomodulatory molecules, called “checkpoints,” include molecules such as cytotoxic T lymphocyte antigen 4 (CTLA-4) and the programmed death receptor 1 (PD-1) on the surface of T cells and its ligand (PD-L1) on tumor cells and cells within the tumor microenvironment. Numerous other checkpoints also exist and may potentially be targeted for therapy (12). Engagement of these specific checkpoints (CTLA-4 and PD-1) results in downregulation of an immune response, and blocking this interaction has resulted in dramatic responses in the treatment of melanoma, and also in the treatment of non-melanoma malignancies (3, 6, 8, 13). These advances led to the FDA approval of several of these agents, including monoclonal antibodies targeting the CTLA-4 (namely Ipilimumab – (6)) and PD-1 axis (Pembrolizumab – (14), Nivolumab – (13)). Though response rates are lower with these therapies (ranging from 15–50 percent), responses tend to be more durable (15). However, a significant proportion of patients do not respond to this form of therapy, and critical insights must be gained to improve responses to therapy.

A critical tool in studying the effectiveness and limitations of these treatment strategies is through the collection and analysis of longitudinal blood and tumor samples during the course of therapy (Fig. 1). Our group has focused on this approach, and together with investigators worldwide, we have analyzed such samples and gained tremendous insight into molecular and immune mechanisms of response and resistance to therapy for both targeted therapy (1) and immune checkpoint blockade (14, 16). Equally important are parallel studies in preclinical and murine models, though it is important to factor in both molecular and immune effects of responses to all forms of therapy in this approach.
Illustrative of these points are the observations regarding the immune effects of molecularly targeted agents. Importantly, genomic alterations present in cancer cells may significantly affect antitumor immunity through modulation of antigen expression and other mechanisms that change the tumor microenvironment. Accordingly, pharmacologic targeting on oncogenic signaling pathways may have a profound impact on antitumor immunity (2). We studied this in melanoma, and demonstrated that treatment of melanoma with BRAF inhibitors or combined BRAF/MEK inhibitors results in a more favorable tumor microenvironment, with increased antigen expression and CD8^+^ T cell infiltrate within two weeks of initiating therapy (17). However, there is a concurrent increase in expression of immunomodulatory molecules such as PD-L1 – suggesting an immune mechanism of resistance (Fig. 2) (17). We have further studied the molecular and immune effects of melanoma therapy, and are beginning to unravel the complex interplay between oncogenic mutations and antitumor immunity. Together, insights have led to the identification of actionable strategies to overcome resistance, such as combining molecularly targeted therapy with immune checkpoint blockade to enhance responses to therapy (2). However, additional insights are needed to fully optimize responses to molecularly targeted therapy and immunotherapy for melanoma and other cancers.

**Future Directions**

The way to optimally move the field of cancer treatment forward in the short term is to better understand response and resistance to cancer therapy through a deep and thoughtful analysis of longitudinal blood and tumor samples during the course of treatment. Such approaches should be built into clinical trials testing novel therapies and combination strategies, as insights gained will help us better understand which patients will benefit from therapy (as well as appropriate timing and sequence of therapy). These analyses should also be strongly considered in patients on standard of care therapy, as we do not yet have a comprehensive understanding as to which patients will benefit from treatment, and we also lack good biomarkers of response and resistance. Analyses should include clinically accepted markers of response and putative markers proposed...
by individual investigators, and should also include a comprehensive deep analysis, if possible, to fully explore novel blood and tissue-based biomarkers that may be useful in guiding treatment. Novel blood-based biomarkers and imaging should be incorporated when available, as there is a critical need to develop these strategies for clinical use to improve care for our patients.

Importantly, such studies may inform (and be informed by) parallel studies in preclinical and murine models. This so-called "co-clinical trials approach" is not novel, and there is increasing evidence that responses to treatment in appropriately chosen models may closely mirror what is observed in human patients (18), providing a window of opportunity to deeply study and characterize responses to therapy to help guide cancer treatment. Through such studies, we may identify biomarkers of response and resistance, as well as actionable strategies to overcome resistance that can be tested in murine models before bringing them forward to human clinical trials.

Another key feature to all these studies is that we must take a more holistic approach in our understanding of the effects of therapy on the entire tumor microenvironment, rather than on cancer cells in isolation. An important component of this is understanding the role of host antitumor immune responses, though it is becoming increasingly apparent that other stromal factors – both locally in the tumor microenvironment (such as stromal fibroblasts and myeloid derived suppressor cells) and distantly (such as the gut microbiome) – may influence responses...
to therapy (19–21). Ultimately, the largest gains will be made through comprehensive studies in patient samples, preclinical studies and murine models, and through extensive collaborative networks to best understand data generated from these studies.

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