Treatment with immune checkpoint inhibitors to relieve immune suppression represents one of the most significant advances in the history of cancer therapy. By mobilizing the immune system against each patient’s tumor, immune checkpoint inhibitors can cause tumor regression or long-term disease control in advanced-stage cancers. In melanoma, immune checkpoint inhibitors targeting cytotoxic T-lymphocyte-associated antigen-4 (CTLA4) or programmed death-1 (PD-1) alone have induced response rates of approximately 10% to 40% and even better efficacy when used in combination (50%–60%), with many of these responses being durable (>2 years; ref. 1). However, there is still a significant proportion of patients with melanoma who do not respond to immune checkpoint inhibitors alone, with toxicities an issue for consideration, particularly when immune checkpoint inhibitors are combined. Given that immune checkpoint inhibitors alone and in combination are FDA approved for the treatment of advanced melanoma, there is an urgent need to identify predictive biomarkers of response and pathways that can be targeted to overcome therapeutic resistance. To date, a number of pretreatment biomarkers have been suggested to correlate with clinical response to immune checkpoint inhibitors, particularly anti–PD-1 therapy. This includes PD-1 and PD-1 ligand (PD-L1) expression on immune cells and tumors (2), preexisting infiltrating lymphocytes, particularly CD8 T cells and their location within the tumor and their effector function (3), as well as tumor-intrinsic mutational load, neoantigens (4), and intratumor heterogeneity (5). Nevertheless, the biomarker profiles between responders and nonresponders can be overlapping, and thus there is a need to identify robust biomarkers of response.

Importantly, an article in this issue by Chen and colleagues suggests that assessment of adaptive immune responses via early on-treatment biopsies should be considered as well as, or in place of, pretreatment biopsies to predict patients who do not respond to immune checkpoint inhibitors alone, with toxicities an issue for consideration, particularly when immune checkpoint inhibitors are combined. Given that immune checkpoint inhibitors alone and in combination (50%–60%), with many of these responses being durable (>2 years; ref. 1). However, there is still a significant proportion of patients with melanoma who do not respond to immune checkpoint inhibitors alone, with toxicities an issue for consideration, particularly when immune checkpoint inhibitors are combined. Given that immune checkpoint inhibitors alone and in combination are FDA approved for the treatment of advanced melanoma, there is an urgent need to identify predictive biomarkers of response and pathways that can be targeted to overcome therapeutic resistance. To date, a number of pretreatment biomarkers have been suggested to correlate with clinical response to immune checkpoint inhibitors, particularly anti–PD-1 therapy. This includes PD-1 and PD-1 ligand (PD-L1) expression on immune cells and tumors (2), preexisting infiltrating lymphocytes, particularly CD8 T cells and their location within the tumor and their effector function (3), as well as tumor-intrinsic mutational load, neoantigens (4), and intratumor heterogeneity (5). Nevertheless, the biomarker profiles between responders and nonresponders can be overlapping, and thus there is a need to identify robust biomarkers of response.

Importantly, an article in this issue by Chen and colleagues involves analysis of a unique set of tissue samples from a cohort of patients with metastatic melanoma initially treated with anti–CTLA4 (n = 53) followed by anti–PD-1 at progression (n = 46; ref. 6). The authors described results from immune profiling of these longitudinal tumor samples, collected at multiple time points during therapy using a 12-marker immunohistochemistry panel and NanoString analysis (Fig. 1A). In these studies, they sought to identify biomarkers of response to CTLA4 or PD-1 blockade and to explore differential changes in the tumor microenvironment caused by these immune checkpoint inhibitors. A key observation is that early on-treatment tumor biopsies following anti–PD-1 from responders (n = 5) showed evidence of an adaptive immune signature that was distinct from and nonoverlapping with nonresponders (n = 6). Another interesting observation was the increased intratumor interaction of CD8+ T cells with CD68+ myeloid cells in nonresponder patients for PD-1 blockade at pretreatment and on-treatment, although no clear quantitative differences in myeloid subsets were observed. Among the responders to anti–PD-1 therapy, there was a profound and highly significant difference in the expression of markers for T-cell subsets (CD8, CD4, and CD3), immunomodulatory receptors (PD-1, PD-L1, and LAG3), and activation status (CD45RO, FOXP3, Granzyme B, and CD57) compared with nonresponders (Fig. 1B) and a trend for increased macrophages (CD68) and B cells (CD20) that was not significant, probably due to the small sample sizes. Importantly, these changes were observed as early as 2 to 3 doses following initiation of anti–PD-1 therapy. In contrast, immune profiling of pretreatment samples largely failed to predict ultimate clinical response. Similarly, analysis of early on-treatment tumor biopsies identified a significantly higher density of CD8+ T cells in responders versus nonresponders to CTLA4 blockade, although the sample sizes were very small (n = 2 for responders vs. n = 3 for nonresponders). Overall, these data suggest that assessment of adaptive immune responses via early on-treatment biopsies should be considered as well as, or in place of, pretreatment biopsies to predict patients who will respond to anti–PD-1 treatment.

Tumeh and colleagues have previously reported that the baseline density and location of CD8+ T cells and PD-1 and PD-L1 expression at the invasive tumor margin and within tumors in metastatic melanomas had predictive value in the treatment outcome of patients receiving anti–PD-1 therapy (3). Critically, they also reported that serially sampled tumors from responders during anti–PD-1 therapy exhibited a further increase in CD8+ T cells at both the invasive...
margin and the tumor center, whereas this increase was not observed in the nonresponders. This current study validates and builds on the Tumeh study and clearly demonstrates that the adaptive immune signatures in tumor biopsy samples obtained early after anti–PD-1 therapy are highly predictive of response. Whereas Tumeh’s study demonstrated the importance of the density of CD8\(^+\) T cells in the invasive tumor margin in predicting response to PD-1 blockade, the report by Chen and colleagues did not observe such differences. This may be due to the small sample sizes in the current study and the fact that a significant proportion of the samples did not have discernible tumor margins. Nevertheless, in samples where such analysis could be performed, Chen and colleagues observed a higher ratio of CD8\(^+\) T cells at the tumor center versus the margin within responders compared to nonresponders, suggesting possible infiltration from margin to the tumor center in the context of therapy. Given that a proportion of responders to PD-1 blockade will display a durable or complete response, it will be interesting to determine if the adaptive immune signature can be further refined to identify these patients, as opposed to those who will eventually develop acquired resistance and progress. Although the early on-treatment biopsies following anti–PD-1 were obtained over a median period of 1.4 months, this represents a range of 0.7 to 26 months. Interestingly, a previous meeting report demonstrated that early on-treatment biopsies (\(n = 21\)) of patients with melanoma within 2 months of commencing anti–PD-1 had a significant influx of intratumoral CD3\(^+\), CD8\(^+\), and CD68\(^+\) macrophages in the responders, but not in the nonresponders (7). It will now be important to set up larger cohort studies to determine how early this adaptive immune signature in responders can be observed, to allow for very early intervention in nonresponders with other strategies. In addition, it will be intriguing to measure for this signature in patients receiving concurrent anti-CTLA4 plus anti–PD-1.

Importantly, this current study also sought to identify putative mechanisms of primary resistance to immune checkpoint inhibitors. When comparing gene expression profiling (GEP) between responders and nonresponders, no

![Figure 1](image-url)
significant differences were observed at pretreatment CTLA4 blockade, on-treatment CTLA4 blockade, and pretreatment PD-1 blockade. However, early on-treatment tumor samples of patients on anti–PD-1 therapy showed hundreds of differentially expressed genes (DEG) in responders, including cytolytic markers, IFNγ pathway effectors, HLA molecules, select adhesion molecules, and chemokines. Interestingly, a small number of DEGs were higher in nonresponders compared to responders on PD-1 blockade and included VEGFA, a molecule potentially involved in therapeutic resistance (6). Other groups have also begun to study mechanisms of therapeutic resistance to immune checkpoint inhibitors, and there is evidence that somatic mutations in antigen processing and presentation and upregulation of genes involved in cell adhesion, angiogenesis, and extracellular matrix remodeling may contribute to immune escape of cancers (8). Furthermore, molecular analyses of human melanoma samples and animal models also suggest that tumor-intrinsic oncogenic signals related to the WNT/β-catenin signaling pathway may mediate cancer immune evasion (8). These data suggest the potential opportunity for targeting with additional immune checkpoint inhibitors or other therapeutic approaches early on treatment.

As immune checkpoint inhibitors such as anti–PD-1 become incorporated into standard of care, monitoring of immune response in the tumor microenvironment should also be incorporated into routine clinical practice to identify patients who will respond, as seen with the screening of BRAF mutations to identify suitable patients. However, there are challenges with current immune monitoring strategies, such as the source of tissue (archival vs. fresh tissue) and the amount available for analysis (limited core biopsy vs. abundant tissue from surgical sample), the collection method (static vs. longitudinal sampling), and immune heterogeneity (highly vs. poorly immune cell infiltrates in tumors; ref. 9). Finally, although biopsies from melanoma are more easily obtained, obtaining posttreatment samples from other solid cancers represents more of a challenge. Nevertheless, there is an imperative to validate if the predictive value of the adaptive immune signature in early on-treatment holds true in other cancer types where anti–PD-1 is more necessary than ever.

In the past five years, cancer immunotherapy utilizing immune checkpoint inhibitors targeting PD-1 has demonstrated unprecedented clinical efficacy in the treatment of advanced cancers. The development of robust biomarkers that can accurately predict complete responders from nonresponders will have a twofold impact. First, it will help identify patients who will respond to monotherapy and thus be potentially spared unnecessary toxicities associated with combination regimes. Second, nonresponders can be treated early and more aggressively with combination therapeutic approaches to give them the best chance of responding. With healthcare costs escalating, picking a winner is now more necessary than ever.

Disclosure of Potential Conflicts of Interest

R. Khanna reports receiving a commercial research grant from Atara Biotherapeutics and is a consultant/advisory board member for the same. M.J. Smyth reports receiving commercial research grants from Bristol-Myers Squibb and MedImmune. No potential conflicts of interest were disclosed by the other author.

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