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33	Keywords separated by ' - '	Immune checkpoint inhibitors - Programmed death-1 (PD-1) - Programmed death ligand-1 (PD-L1) - Exhausted T cells (Tex) - Tumor microenvironment (TME) - Memory T cells (Tmem) - Tumor mutation burden - Circulating tumor DNA
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MELANOMA (RJ SULLIVAN, SECTION EDITOR)

Prognostic Biomarkers for Melanoma Immunotherapy

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Abstract

Purpose of Review Recent developments in immunotherapy have transformed the landscape of melanoma therapy. Here, we review markers for response to immunotherapy.

Recent Findings Current immunotherapies disable immune checkpoints on T cells and other immune cells and allow immune rejection of tumor. This process depends crucially on a preexisting response to the development of the melanoma. Here we describe the complexity of the anti-tumor immune response and the links to the development of markers that are currently used or under investigation in the clinic.

Summary We describe immune response biomarkers along with new developments that could translate into advances.

Keywords Immune checkpoint inhibitors · Programmed death-1 (PD-1) · Programmed death ligand-1 (PD-L1) · Exhausted T cells (Tex) · Tumor microenvironment (TME) · Memory T cells (Tmem) · Tumor mutation burden · Circulating tumor DNA

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Introduction

Melanoma treatment has been transformed recently by the development of rapidly accelerated fibrosarcoma (RAF) and mitogen-activated protein (MAP) kinase inhibitors [1–4] and by immune checkpoint Inhibitors (CPI) such as anti-PD-1 and anti-CTLA-4 [5], with many patients deriving long-term clinical benefit [1, 3, 6]. However, these durable responses still occur only in a fraction of patients and can be associated with significant toxicity, particularly when used in combination. In this review, we focus on our current understanding of the mechanism of action of immunotherapy and on biomarkers to select patients for treatment on clinical trials and for particular therapies in the clinic.

The stages of neoplastic transformation and associated molecular alterations have been well described for melanoma [7–9], yet complex tumoral/stromal/immune interactions result in tumor heterogeneity that is evident in patients with the same histological signatures as well as between tumors within the same patient and even within different areas of a single tumor [10–12]. The mechanisms fundamental to CPI and specifically to anti-PD-1/PD-L1 activity in a varied and often complex tumor microenvironment (TME) have led to the identification of a multifactorial process dependent on the interactions of specific cell types with diverse functions.

Current Understanding of the Mechanism(s) of Action of PD-1 Check Point Inhibitors

Tumor immunogenicity or the ability of the tumor to trigger a productive immune response is arguably fundamental to all effective anti-cancer therapies, including some chemo/radio therapies and targeted therapies but especially immunotherapies. While melanoma is widely recognized as an immunogenic tumor overall, great variability in immunogenicity is evident during disease progression and between patients or even lesions, which may shed light on the effectiveness of anti-PD-1 therapies [13]. Ultraviolet radiation coupled with key molecular changes which are the primary drivers in the

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malignant transformation of melanocytes often produces an exceptionally high rate of somatic mutations [7, 14]. These mutations which promote tumorigenesis by coordinated dysregulation of cellular processes are also central to its immunogenicity with the emergence of neoantigens, as well as increased cancer/testis or differentiation antigens [15]. Importantly, immunogenicity is not static; indeed, co-evolution of the immune system with the tumor initiates before neoplastic transformation. Immune pressure either eliminates developing tumors or steers them towards an equilibrium and ultimately to tumor escape by immune evasion as well as by direct and indirect subversion of the immune response itself [16, 17]. The process of immunoediting highlights not only the dynamic nature of immunogenicity but also provides insight into the complexity and evolving spatial/temporal interplay between the tumor and immune response, which lies at the heart of the effectiveness of CPI immunotherapy.

Activated lymphocytes including NK and T cells transiently express PD-1 on their cell surface, which in the melanoma setting may represent recently engaged tumor-specific T cells. Significantly, translational analysis of adoptive cellular therapy patients has identified that PD-1+ rather than PD-1− CD8+ tumor infiltrating lymphocytes (TIL) conferred superior oligoclonal expansion of tumor-reactive TCRβ clonotypes, suggesting that PD-1 expression may mark a population of anti-tumor CTL [18]. However, chronic TCR signaling can lead to sustained PD-1 expression and the triggering of immune adaptation, a physiological reaction to curb an inappropriate or autoimmune response that can be usurped by tumor cells to promote peripheral tolerance. Sustained PD-1 expression along with increasing co-expression of additional markers such as CTLA-4, TIM-3, LAG-3, TIGIT, and VISTA denotes the transition from an activated effector (Teff) to an exhausted T cell (Tex). In a typical melanoma setting replete with chronic antigen stimulation, T cells tend to exist on a continuum that ranges from a state of stemness towards dysfunction with effector or memory-like states being key intermediates. Identification and interrogation of these progressively differentiating T cell subsets are paramount to appreciating an effective anti-PD-1 therapy [19, 20].

The use of a single or limited set of markers is likely to be inaccurate in discriminating between transitioning immune populations as some markers such as PD-1 are shared between immune subsets. Instead, linked functional characteristics can more readily delineate these discrete intratumoral states with a transition from high levels of cytotoxic molecules (granzymes/perforin) and effector cytokines (Interferons, IL-2, IL-12, and TNF), a high proliferative capacity and anabolic metabolism associated with an effector subset to limited or absent cytotoxic/effector molecules, low proliferative capacity, and catabolic metabolism associated with dysfunctional or highly exhausted T cells [21]. Additionally, specific transcription factors, gene expression profiles, and epigenetic signatures yield an even finer picture of these subsets while providing insight into their respective functions [19]. T-bet, a transcription factor associated with Th1-biased response, classically associated with Teff cells also plays a role in Tex cells [22]. The transcription factors NR4A, EOMES, and TOX all have been associated with a Tex lineage while TCF1 additionally drives a stem-like progenitor lineage capable of self-renewal, while seeding Teff cells and memory T cells (Tmem) cells [19, 23, 24]. Recent advances in transcriptomic (scRNA-seq) and epigenetic (ATAC-seq) analyses effects of anti-PD-1 blockade have identified gene and epigenetic signatures associated with these subsets along with their key regulators [25•, 26].

Understanding the functional role that these discrete T cell populations play during PD-1 blockade continues to be a high priority with the initial focus being on exhausted T cells. Interestingly, a high frequency of tumor infiltrating CD8+ T cells expressing PD-1+/CTLA-4+, a subset of exhausted T cells is highly predictive of anti-PD-1 CPI response in metastatic melanoma patients [27•, 28]. Conversely, a low intratumoral frequency of these Tex cells exhibited a negative predictive value with single-agent anti-PD1 therapy yet this low frequency was not associated with poor outcomes in patients treated with combination anti-CTLA-4 and anti-PD-1, suggesting a non-redundant mechanism with the combination CPI [28]. Similarly, circulating peripheral Tex cells (PD-1+/CTLA-4+ CD8+ T cells) were found to be "reinvigorated" during anti-PD-1 therapy of melanoma, which was associated with a positive clinical outcome particularly in patients with a larger ratio of proliferating Tex cells to tumor burden, defined as the sum of the long axis of all measurable lesions reported on the pre-therapy imaging reports [29•]. While these studies suggest that tumor-reactive Tex are a major target of PD-1 CPI with functional roles both in the TME and systemically, a chronic LCMV model demonstrated that the anti-PD-1 reinvigoration is transient, and exhaustion persists shortly after PD-1 CPI treatment due to a stable epigenetic signature [25•,30]. This functional maintenance of exhaustion may account for the narrow proliferative burst of Tex when on treatment and clarify its relationship with tumor burden; beyond the clear prognostic consideration, a larger tumor burden can more easily withstand the effector response associated with a transient proliferative burst while readily providing chronic antigenic stimulation in addition to PD-L1 or other inhibitory immune signals in the TME required to maintain T cell exhaustion. The dysfunctional state associated with Tex epigenetic program, particularly in the $\text{Eomes}^{hi}\text{PD-1}^{hi}$ subset, is unable to sustain a memory-like response with PD-1 blockade, which is critical for effective tumor immunity and remains distinct from naïve T cells (Tnaive), Teff, and Tmem cells [25•, 26]. The fate of Tex cells underscores that while exhausted T cells can be useful to predict response and can certainly contribute to the efficacy of PD-1 blockade when

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tumor burden is low or if PD-1 blockade occurs before reaching a late dysfunctional state, immune subsets capable of self-renewal and persistence in the presence of chronic antigen are likely required for sustained responses.

The necessary role of Teff cells in a productive anti-tumor immune response has been well documented but the cell types that maintain this pool of effectors while enabling memory-like subsets continue to be defined. Expression of the transcription factor TCF-1 in T cells has identified a self-renewing precursor population critical for response to immunotherapy both in preclinical models and in patients with melanoma [31, 32]. In a chronic LCMV model, IL-12 or other inflammatory mediators could blunt TCF-1 expression via STAT-4, allowing for the differentiation of KLRG1+ Teff [33]. However, TCF-1 in a PD-1-dependent fashion could suppress TCF-1− T-bet+/KLRG1+ Teff differentiation while establishing a CD8+ Eomes+ Tex precursor population [24]. The factors underlying the generation, maintenance, or fate choices of these stem-like TCF-1+ T cells are actively being investigated. A recent study has revealed that the metabolic state of the TME, specifically elevated extracellular potassium which induces a starvation response/autophagy and catabolic metabolism, promotes stem cell–like TCF-1 expressing T cells via an epigenetic-dependent stemness-associated program [21].

Other studies have demonstrated innate immune mechanisms that can also be instrumental in effective CPI therapies. PD-1 is expressed on natural killer (NK) cells and when engaged with PD-L1 can limit NK cytotoxicity. In tumors with loss of MHC class I, rejection was dependent on these innate effectors which was significantly enhanced with PD-1 blockade [34]. Even in models where CD8+ T cells routinely mediate tumor regression, PD-1+ NK cells demonstrated a meaningful contribution to anti-PD-1 therapy with notable selection of PD-L1+ tumor cells [34]. Further, NK cells were shown to play a significant role in the efficacy of PD-1 blockade in melanoma by producing the cytokine FLT3L and forming stable conjugates with CD141+ cDC1, resulting in increased frequencies of the DCs [35]. Similarly, cDC1 subsets were shown to play an additional key role in the efficacy of PD-1 blockade by producing IL-12, triggering IFN-γ secretion from PD-1+ T cells, which further engaged IL-12 secretion from DCs. The resulting IL-12/IFN-γ feed-forward loop which initiated with anti-PD-1 treatment helped further license theses PD-1+ T cells and enhance PD-1 blockade [36]. A separate study demonstrated the role of benefit in PD-1 blockade of PD-1+ on CD103+ DCs that engage and activate intratumoral T cells via production of CXCL9/CXCL10 [37]. Conversely, increased oncogenic, β-catenin signaling in TME leads to downregulation of CCL4, which blunts the frequency of a similar subset of DCs, ultimately limiting T cell recruitment [38, 39]. Beyond NK and DC subsets, PD-1+ tumor-associated macrophages (TAM) with an M2-like phenotype, which generally are associated with poor patient outcomes, can contribute to anti-tumor immunity via tumor phagocytosis when in the presence of anti-PD-1 in combination with other therapeutics like anti-CSFR1 or anti-SIRPα blocking antibodies [40, 41].

The mechanisms fundamental to CPI and specifically anti-PD-1/PD-L1 activity in a varied and often complex tumor microenvironment (TME) have led to the identification of a multifactorial process dependent on the interactions of specific cell types with diverse functions. While this complexity can pose a challenge to identify the relevant parameter(s) specific for a given patient or even a specific lesion, many of these seemingly distinct mechanisms converge on the TME with specific immune subsets driving a collective immunogenicity in turn creating a foundation for effective anti-PD-1 therapy.

Biomarkers of Response to CPI

PD-L1 Expression

Following the discovery of PD-1 expression on lymphocytes [42], the B7 family member, B7-H1, was identified as the ligand for PD-1 [43]. This protein, called B7-H1, was identified by Dong et al. based on its similarity to the co-stimulatory ligands B7-1 and B7-2 on immune cells [44]. Ligation of this protein in the context of antigen binding on T cells caused IL-10 secretion. The pattern of expression of B7-H1 in malignant neoplasms such as melanoma was very interesting. Notably, B7-H1 was strongly co-localized with tumor infiltrating lymphocytes [45•]. In addition, interferon-γ was found at the interface of B7-H1 expressing tumor cells and TILs. In this same study, B7-H1 (now more commonly referred to as PD-L1) was also found to be a prognostic marker, predicting extended survival. When tumor tissue was analyzed in patients treated with the PD-1 blocking antibody, nivolumab, PD-L1 expression was found to correlate with response [46]. A similar observation was made with the PD-L1 antibody, atezolizumab, in a phase I multiple solid tumor trial [47]. These observations were confirmed with pembrolizumab [48].

Given the clinical and translational data supporting the use of PD-L1 as a biomarker for response to PD-1 blockade, many recent trials have explored this prospectively and retrospectively. In a retrospective analysis of patients treated on the Keynote 001 trial, tumors from 451 patients (out of 655 patients treated) were stained with the 22C3 monoclonal antibody [48]. Samples were assessed by a quantitative membranous staining called the MEL score which incorporated intensity and frequency of staining on tumor and tumor adjacent stromal and immune cells. A positive score was anyone with MEL ≥ 2 (staining in $\geq 1\%$ of cells). Of the 451 patients evaluated, 344 (76%) had PD-L1-positive tumors. A higher MEL score was associated with a higher response rate and longer

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PFS (hazard ratio, 0.76; 95% CI, 0.71 to 0.82) and OS (hazard ratio, 0.76; 95% CI, 0.69 to 0.83) ($P < .001$ for each). The objective response rate was 8% for MEL 0 and ranged up to 57% for MEL 4 showing the dynamic range of this marker. In a prospective nivolumab vs dacarbazine clinical trial [49], using the rabbit monoclonal 28-8 antibody, 5% or greater tumor cell staining was considered "positive" [50] with 2 pathologists independently scoring using an automated Dako stainer. In the PD-L1-positive group, 52.7% had an objective response to nivolumab versus 33.1% in the PD-L1-negative group. Other trials in melanoma have consistently shown a higher response rate and higher PFS (and in some cases a higher OS) in PD-L1 high patients [6, 51–53]. Some of the questions that remain with PD-L1 IHC have to do with the difference between tumor and/or stroma and different monoclonal antibodies although recent data has shown that most widely used PD-L1 monoclonal antibodies are quite consistent, reproducible, and inter-comparable regardless of the specific methodology used in the hands of trained experienced pathologists using recent rapidly processed specimens [54]. Also, PD-L1 expression has differing predictive value in malignancies arising from differing sites. In non-small cell lung cancer, in the Keynote 001 clinical trial, the response rate of pembrolizumab varied from 8.1% in the < 1% PD-L1 group to 29.6% in the PD-L1 50–74% group [55]. In Keynote 010, where chemo-naïve patients were randomized to pembrolizumab at 2 mg/kg or 10 mg/kg (or docetaxel), PD-L1 staining of 1–49% was associated with a response rate of 10% while ≥ 50% PD-L1 had a response rate of 30% (all for the pembrolizumab cohort). Contrast these findings to nivolumab in renal cell cancer in the Checkmate 025 study where PD-L1 expression was not found to significantly predict benefit from PD-1 blockade (< 1%, OS was 27.4 months while PD-L1 ≥ 1%, OS was 21.8 months). Similar results were seen in Keynote 427, which examined first line pembrolizumab in renal cell cancer; no difference in response rate with PD-L1 expression [56].

Immune Cell Infiltration and "Exhausted" T (Tex) Cells in the Tumor Microenvironment

Tumor infiltrating lymphocytes (TIL) have been shown to correlate with prognosis in melanoma (as well as in many other tumor types) [57–59]. An important study, by Tumeh et al., showed that CD8+ TIL density in tumor samples was higher in responding patients than in patients with disease progression [60•]. Subsequently, it was demonstrated that CD8+ cells with dual PD-1/CTLA4 expression, or "exhausted" CD8+ cells, were predictive of PD-1 monotherapy response in melanoma [27•]. Furthermore, lower levels of "exhausted" CD8+ cells were associated with response to dual PD-1/CTLA4 inhibitor therapy but were insufficient for monotherapy PD-1 blockade [28]. Another important study by Broz et al. showed that the presence of cDC1 dendritic cells in melanoma was predictive of response [61•]. These findings have been extended by Spranger et al. who showed that BATF + dendritic cells are characteristic of immune infiltrated melanoma [62]. More recently, the Krummel group demonstrated that there are 2 axes in "immunogenic" tumors, one a NK-cDC1 axis [35] that operates in checkpoint responsive tumors and a CD4-cDC2 axis [63]. Other axes may operate in other tumor types as well.

Recent translational studies have shed light on the biology, location, and surroundings of Tex cells. Tex have a distinctive transcriptional profile that is maintained stably via large-scale epigenetic programming and transcription factors [25•, 30]. The presence of Tex cells in tumors can predict responses once tumor burden is factored in [29•]. Thommen et al. reported that these cells produce chemokines that attract B cells and TFH cells that produce tertiary lymphoid structures [64]. More recently, the transcription factor Tox, highly expressed in exhausted T cells, appears to be critical to maintain their tissue presence but not the dysfunction associated with them [65–67]. There remain unanswered questions about precursor and terminal exhausted T cells and the transitions possible between these states [68].

Tumor Mutation Burden and MSI

Tumors with high mutation burdens appear to have an increased response rate and better survival in response to PD-1 immunotherapy [69]. In non-small cell lung cancer, a higher non-synonymous mutation burden was associated with better PFS, OS, and objective response when treated with anti-PD-1 [70]. While some neoantigens are clonal (shared by multiple sites), others are present in a more localized fashion (branch). It has been hypothesized that because of the selection pressure that neoantigen-directed T cells put on tumor cells, that non-clonal neoantigen bearing tumor cells could be edited out while clonal neoantigens persist and can predict for greater response to PD-1 inhibitors [71]. The use of tumor mutation burden to select patients for PD-1 therapy has not yielded consistent results and at present is experimental [72]. These studies need additional replication to be widely accepted. At present, it is unclear how tumor mutation burden impacts melanoma although it has been noted that uveal melanoma, which has a low tumor mutation burden has a low response rate to PD-1 blockade [73, 74].

Extremely high rates of tumor mutation burden are seen in patients with mismatch repair deficiency [75]. These tumors also have a very high response rate to PD-1 immunotherapy [76, 77] and anti-PD-1 therapy is approved for use in these patients regardless of primary site. Widespread and continual mutation resulting from a deficiency in DNA repair is thought to generate neoantigens which in turn prime T cells [78]. While melanoma is not part of Lynch syndrome and mismatch

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repair deficiency is uncommon in melanoma, desmoplastic melanoma, which can have a high UV mutagenesis signature, is also associated with a high response rate to anti-PD-1 therapy [79, 80]. Basal cell cancer of the skin is the most mutated non-mismatch repair deficient cancer [81]. Squamous cell cancer of the skin also has a very high mutation burden [82]. Squamous cell cancers respond well to PD-1 blockade, while response rates for basal call cancers are lower [83, 84]. Merkel Cell Cancer, an uncommon skin neoplasm, also responds to PD-1 blockade regardless of polyoma viral status [85] although only the merkel cell polyoma virus negative tumors have a high mutation burden, presumably due to UV damage [86]. These data illustrate the complexity of this field and the continuing research into the impact mutation burden has on PD-1 response.

Peripheral Blood Biomarkers

There is great interest in identifying peripheral blood biomarkers associated with favorable response to immunotherapy in melanoma, as these could be serially collected and offer significant safety, cost, and convenience advantages. Peripheral biomarkers could also allow for profiling of the systemic immune response in a way that tumor biopsies cannot.

Since basic peripheral blood laboratory variables are collected routinely in standard clinical care, it is possible to study these variables in large retrospective clinical studies. For example, Martens et al. analyzed peripheral blood biomarkers of 209 patients with advanced melanoma on ipilimumab. They found that a baseline signature of low lactate dehydrogenase (LDH), absolute monocyte count (AMC), and myeloid-derived suppressor cells (MDSC), as well as high absolute eosinophil count (AEC), regulatory T cells, and relative lymphocyte count (RLC) were associated with improved outcomes with ipilimumab therapy [87]. Similarly, Weide et al. analyzed peripheral blood biomarkers of patients with advanced melanoma treated with pembrolizumab and found that high relative eosinophil count (REC), high relative lymphocyte count (RLC), low LDH, and absence of metastasis other than soft-tissue/lung metastases are independent baseline characteristics associated with favorable overall survival [88]. Most recently, Rosner et al. evaluated peripheral blood clinical laboratory variables associated with outcomes following combination nivolumab and ipilimumab immunotherapy in melanoma. They found that significant independent variables for favorable OS included the following: high relative eosinophils, high relative basophils, low absolute monocytes, low LDH, and a low neutrophil-to-lymphocyte ratio [89]. Further work is needed to validate these peripheral blood biomarkers in randomized controlled clinical trials. Ultimately, such biomarkers could be a simple and cost-effective way to define which patients with metastatic melanoma may derive the most benefit from immunotherapy.

Immunotherapy and the Microbiome

Complex microbial communities, known as the microbiota, colonize the mammalian host and contribute to the health of the host [90]. Over the last few decades, there has been increasing evidence to suggest that the bacterial microbiome plays an important role in carcinogenesis as well as the body's response to cancer treatment [91] [92]. While CPI therapy has revolutionized the treatment of metastatic melanoma, response to CPI therapy is variable, with some patients achieve a robust response while other patients have minimal or no response. One hypothesis that has emerged recently is that the gut microbiome may affect response to CPI therapy, and thus the study of the gut microbiome can yield important clues about which patients will derive the most benefit from immunotherapy.

There is evidence in mouse models that modulation of the gut microbiome may enhance responses to immune checkpoint blockade, so several groups have studied whether the human microbiome affects response to CPI therapy. In a study by Gopalakrishnan et al., the authors examined the oral and gut microbiome of 112 melanoma patients undergoing anti-PD-1 immunotherapy [93]. The authors observed significant differences in the diversity and composition of the gut, but not oral, microbiome of patients who responded to PD-1 therapy versus those patients who did not respond, namely responders had higher alpha diversity ($P < 0.01$), relative abundance of bacteria of the Ruminococcaceae family ($P < 0.01$), and showed significantly higher alpha diversity ($P < 0.01$) and relative abundance of bacteria of the Ruminococcaceae family ($P < 0.01$) in responding patients. In a similar study analyzing the stool microbiota before and after anti-PD-1 therapy, Matson et al. showed that patients who responded to anti-PD-1 therapy had an abundance of certain bacteria, including *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* compared with non-responders [94]. One hypothesis is that the increased bacterial diversity in some patients leads to increased immune cell infiltration. Wargo et al. performed immune profiling of stool samples from melanoma patients and demonstrated increased tumor immune infiltrates in responding patients, with a higher density of CD8+ T cells which correlated with abundance of specific bacteria enriched in the gut microbiome [95]. Together, these studies suggest that the commensal microbiome of patients may have a mechanistic impact on anti-tumor immunity. Further studies are needed to better understand the precise mechanisms mediating this effect, and specifically to determine whether there are ways to modulate the microbiome to affect response to treatment.

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468 Aside from the ways in which the gut microbiome affects 469 response to therapy, another interesting observation is that the 470 gut microbiome may influence which patients are most at risk 471 for checkpoint blockade-induced colitis. In a prospective 472 study of patients with metastatic melanoma undergoing 473 ipilimumab treatment, authors correlated the pre-474 inflammation fecal microbiota and microbiome composition 475 with subsequent development of colitis [96]. They observed 476 that patients with a paucity of bacteria involved in polyamine 477 transport and B vitamin biosynthesis was associated with an 478 increased risk of colitis, whereas patients with increased rep-479 resentation of bacteria in the Bacteroidetes phylum were more 480 resistant to the development of colitis.

481 Conclusions

482 Recently, we have seen a rapid increase in our understanding 483 of the mechanism of action of CPI. Melanoma has served as a 484 model system for many functional and analytical studies. 485 While some of these laboratory advances have translated into 486 clinical and translational studies, in many instances, the com-487 plexity of the immune response to tumor has stymied attempts 488 to develop markers that accurately and comprehensively pro-489 file the immune response to tumor. While it is unlikely that a 490 single biomarker or a simple combination of biomarkers can 491 provide the profile we need as clinicians and translational 492 researchers, rapid advances are underway and we expect some 493 of these advances to translate into trial and clinical use.

494 Compliance with Ethical Standards

495 **Conflict of Interest** Christopher G. Twitty declares that he has no con-496 flict of interest. Laura A. Huppert declares that she has no conflict of 497 interest. Adil I. Daud has received research funding from Merck, 498 OncoSec, Bristol-Myers Squibb, Incyte, Checkmate, and Pfizer.

499 **Human and Animal Rights and Informed Consent** This article does not 500 contain any studies with human or animal subjects performed by any of 501 the authors.

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503 Papers of particular interest, published recently, have been 504 highlighted as:
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