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Predictive and on-treatment monitoring biomarkers in advanced melanoma: Moving toward personalized medicine

Ahmad Tarhini[a,*], Ragini R. Kudchadkar[b]

[a] Cleveland Clinic Taussig Cancer Institute, Cleveland, OH, United States
[b] Winship Cancer Institute of Emory University, 1365 Clifton Rd NE, Bldg C, Atlanta, GA 30322, United States

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ABSTRACT

The treatment armamentarium for patients with metastatic melanoma has increased substantially over the past decade with the regulatory approval of targeted BRAF + MEK inhibitors and immune checkpoint inhibitors, which have vastly improved long-term outcomes. Recently, these advances have been rapidly translated to the high-risk adjuvant setting. Primary and acquired resistance to both immune and molecularly targeted agents, however, remains a challenge. Therefore, biomarkers predictive of response to therapy that can be assessed prior to initiation of treatment and early during the course of therapy are critical. Equally important is on-treatment biomarker monitoring that may predict the likelihood of treatment failure and disease relapse. This review will summarize recent advances in the understanding of biomarkers for patients with advanced melanoma, emphasizing emerging baseline predictive factors and on-treatment monitoring of biomarkers that aim to establish truly personalized treatment.

Introduction

Melanoma incidence is increasing globally, with 351,880 cases reported in 2015 [1]. In the United States alone, an estimated 91,270 new cases and 9320 deaths were predicted in 2017 [2]. The treatment of unresectable or metastatic melanoma has been transformed by the introduction of novel molecularly targeted and immune therapies [3–6]. The discovery of driver oncogenes has facilitated the clinical development of targeted therapies, ushering in the era of personalized medicine. In melanoma, this has been exemplified by the success of agents targeting BRAF and downstream MEK proteins in patients with activating *BRAF* mutations [7–11]. Furthermore, immune checkpoint inhibitors, including anti–cytotoxic T-lymphocyte–associated antigen 4 (anti–CTLA-4) and anti–programmed death 1 (anti–PD-1) inhibitors, have demonstrated substantial benefit as treatment options for patients with melanoma, regardless of their oncogenic driver mutation status [12–14]. For example, recent 5-year overall survival (OS) results from a phase 2 trial of patients with *BRAF* V600–mutant unresectable or metastatic melanoma treated with dabrafenib + trametinib demonstrated a median OS of 25.0 months and a 5-year OS rate of 28% in patients receiving the approved label dose [15]. Similar results have been observed in patients treated with anti–PD-1 monotherapy or a combination of anti–PD-1 and anti–CTLA-4 agents. A recent OS analysis of the phase 3 KEYNOTE-006 trial showed a 33-month OS rate of 50% in patients receiving pembrolizumab monotherapy [16]. In the phase 3 CheckMate 067 trial, patients treated with nivolumab monotherapy or nivolumab + ipilimumab had 3-year OS rates of 52% and 58%, respectively [14].

Despite the increases in 5-year OS with these new agents, many patients still do not achieve long-term disease remission and control [14,15]. Primary and acquired resistance remains a major barrier to successful melanoma treatment and, ultimately, long-term remission. Patients are considered to have primary resistance to therapy when no clinical benefit is observed following treatment. This form of resistance also includes hyperprogressive disease in which patients experience ≥2-fold increase in tumor growth rate and worsening clinical status [17–19]. Acquired resistance differs in that disease progression occurs after a period of tumor response. Although the rate of primary resistance to targeted agents in patients with *BRAF* V600–mutated melanoma is very low, approximately half of patients will develop acquired resistance to combination BRAF + MEK inhibition within 9–12 months [10,11]. Conversely, although immune checkpoint inhibition can produce durable outcomes in some patients, the rate of primary resistance within the first 6 months is relatively high [20,21].

Understanding the biology behind these clinical outcomes will be key to personalized therapy. It is paramount that clinicians determine the optimum treatment for each patient, using biomarkers that may guide the targeting of a specific therapeutic agent toward those who

* Corresponding author at: Cleveland Clinic Taussig Cancer Institute, 9500 Euclid Ave., Cleveland, OH 44195, United States.
E-mail address: tarhina1@ccf.org (A. Tarhini).

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have the capacity to respond while saving others the burdens of unwanted toxicities and costs in the absence of predicted benefit. At present, validated biomarkers predictive of response to available therapies are limited to *BRAF* mutation status and remain a critical need to aid in treatment selection [22]. Emerging technological advances combined with a growing knowledge of tumor biology present the opportunity to evaluate on-treatment markers that could help to predict outcomes, provide an early indication of response or progression, and aid in the understanding of therapeutic resistance.

The present review will summarize recent advances in the understanding of biomarkers for melanoma, with an emphasis on emerging baseline predictive biomarkers and on-treatment monitoring that could help shape the future of personalized melanoma treatment.

Biomarkers in melanoma

Biomarkers may be either prognostic or predictive in nature (Fig. 1). A prognostic biomarker provides insight into the overall disease outcome of a patient but does not predict the likelihood of benefit from a treatment. Predictive biomarkers, on the other hand, provide insight into the probability of therapeutic response of a patient's disease to a particular treatment.

The earliest clinical markers that helped to inform prognosis were based on baseline clinical characteristics, including serum levels of lactate dehydrogenase (LDH), a marker of tumor burden [23]. In its role to catalyze pyruvate into lactate, LDH can be a marker of cancer metabolic activity and increased glucose uptake by tumor cells highly dependent on the anaerobic glycolytic pathway [24]. LDH has been shown to be a negative prognostic marker, regardless of treatment received, even with modern-age therapies [15,25–28]. Elevated LDH is traditionally associated with poor OS compared with normal LDH and is an important marker in determining staging of patients with distant metastases in the American Joint Committee on Cancer (AJCC) staging system of melanoma [29]. Of the approximately 30% of patients with long-term survival (4–5 years following treatment initiation) on BRAF + MEK inhibitors, few had high LDH prior to starting therapy [15]. Similar to serum LDH, levels of S100B in the serum have consistently demonstrated prognostic value in both the metastatic [30,31] and high-risk resected settings [32]. Gene expression profiling, while still in its infancy, has shown some validity as a prognostic biomarker assay that may complement existing techniques in patients with melanoma [33–35].

Biomarkers predictive for response to therapy aid in clinical decision making and provide an evidenced-based rationale to inform treatment decisions. The most notable and well-characterized predictive biomarker in patients with metastatic melanoma is the presence of a *BRAF* V600 mutation, which is highly predictive for response to BRAF ± MEK inhibition with low rates of primary resistance [7,8,11]. Response to BRAF + MEK inhibitors is approximately 70% in selected patients, with < 10% of patients having a best response of progressive disease [11,36–38].

Additional oncogenic driver mutations have been described that could be predictive of benefit from targeted agents (eg, *NRAS*, *NF-1, c-KIT*) [39–43]. Nevertheless, *BRAF* V600 mutation remains the only validated predictive marker for patients with melanoma; however, several other markers are currently being evaluated in clinical studies that may one day help inform treatment decisions.

Emerging predictive biomarkers

Evidence is emerging for the potential predictive value of several biomarkers for response to or progression on either targeted therapy or immune checkpoint inhibition.

Targeted therapy

Multiple baseline analyses have demonstrated that patients with *BRAF* V600E mutations often have concomitant molecular alterations in other genes that may predict response to therapy [44,45]. For example, overall mutation burden, which has been linked to neoantigen formation and enhanced tumor immunogenicity [46], was associated with longer OS in patients treated with dabrafenib + trametinib and a trend toward longer OS in patients treated with vemurafenib + cobimetinib [44,45]. In terms of specific genes, mutation and deletion of the tumor suppressor gene *CDKN2A* were significantly associated with poorer OS and progression-free survival (PFS) in patients treated with dabrafenib + trametinib [44]. Two further potential markers were identified in the coBRIM trial of vemurafenib + cobimetinib: immune response

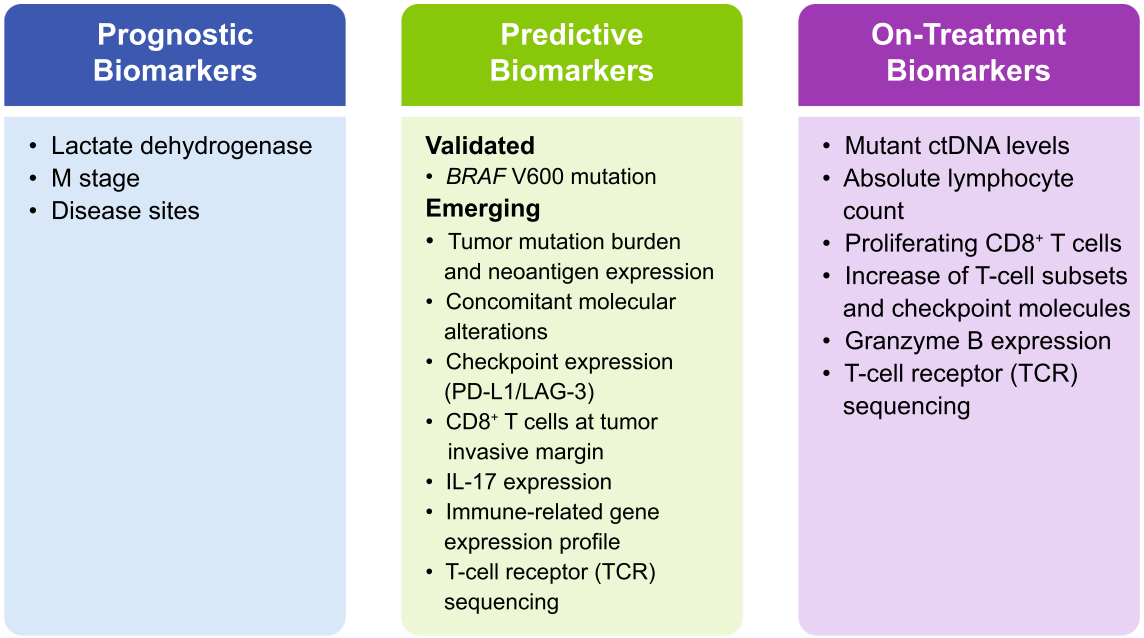

Fig. 1. Prognostic, predictive, and on-treatment biomarkers in metastatic melanoma. ctDNA, circulating tumor DNA; IL, interleukin; LAG-3, lymphocyte-activation gene 3; PD-L1, programmed death-ligand 1.

gene clusters were associated with complete response while keratinization genes were associated with intrinsic resistance [45].

Immune checkpoint inhibition

The immune–cancer interaction is pivotal to the function of immune-based antitumor therapies such as immune checkpoint inhibitors. A number of factors within the tumor and the surrounding microenvironment can influence the ability of the immune system to recognize the tumor and mount an appropriate antitumor response [47]. These factors may also influence the effectiveness of immune checkpoint inhibitors, and recent research has elucidated a number of potential predictive biomarkers based on immune features of the tumor and microenvironment [47].

Overall mutation burden and tumor recognition

Similar to the aforementioned observations in patients treated with targeted therapy, response to immune checkpoint inhibitors is highest among tumor types with a high mutation load (eg, melanoma and lung cancer) [46,48]. This may be attributable, at least in part, to the production of tumor-specific neoantigens. Mutations within a tumor may lead to the formation of peptides unique to tumor cells that have the potential to be antigenic. Therefore, an increase in the mutational load of a tumor could increase the likelihood of production of antigenic tumor-specific peptides, in turn leading to a larger pool of tumor-specific T cells. This larger pool of tumor-specific T cells would theoretically produce a greater antitumor response on inhibition of immune checkpoints that may be mediating tumor immune tolerance [46]. Deficient DNA mismatch repair (dMMR) and high microsatellite instability (hMSI) are validated biomarkers of response for all tumor types and are associated with poor prognosis following conventional chemotherapy in patients with colorectal cancer [49,50]. Levels of immune checkpoint proteins are greater among patients with colorectal cancer with dMMR vs proficient DNA mismatch repair (pMMR) [51]. Recent data suggest durable responses can be obtained with PD-1 blockade in patients with dMMR/hMSI colorectal cancer [52].

Immune checkpoints and associated ligands

PD-L1 expression

The potential association between PD-L1 expression and response to anti–PD-1 therapy was first observed in a study of 39 patients with solid tumors treated with nivolumab monotherapy, in which 3 of 4 patients with surface programmed death-ligand 1 (PD-L1) expression had a response, and no clinical response was observed in 5 patients with PD-L1–negative tumors [53]. This striking finding was investigated further and, subsequently, enhanced efficacy was observed in phase 2 and phase 3 trials with pembrolizumab and nivolumab monotherapy among patients with PD-L1–positive vs PD-L1–negative tumors, although the definition of PD-L1 positivity varied across studies [14,54]. Notably, it has been repeatedly demonstrated that the likelihood of response increases with increasing PD-L1 expression [14,54]. In a descriptive comparison, combination nivolumab + ipilimumab showed a trend toward better OS vs nivolumab monotherapy in patients with low PD-L1, but OS was similar between the 2 arms among patients with PD-L1 ≥ 1% or ≥5% [14]. Increased tumor PD-L1 expression has also been associated with improved outcomes in patients with non-small cell lung cancer who are treated with anti–PD-1 antibodies. The KEYNOTE-024 trial enrolled only patients with ≥50% tumor proportion score and results from this trial demonstrated a significant OS benefit favoring pembrolizumab vs chemotherapy in this population [55]. Despite the association between PD-L1 expression and efficacy of anti–PD-1 agents, responses are observed in patients with PD-L1–negative tumors, limiting the utility of tumor PD-L1 expression as an absolute predictive biomarker [54,56]. Nevertheless, PD-L1 expression has the potential to provide useful guidance in treatment decision making if the appropriate caveats are considered. First, PD-L1 expression may be heterogeneous within tumors, and discordance in PD-L1 expression has been observed between primary melanoma sites and distant metastases. Together, these findings suggest that a single biopsy may not provide an accurate characterization of the overall PD-L1 status of metastatic melanoma [57]. Furthermore, it should be noted that PD-L1 expression is likely to be dynamic and could be influenced by factors such as treatment received postbiopsy. There also remains no consensus on the assay to be used for testing or the appropriate threshold to determine positive status [58]. In an effort to define a cutoff, the recently developed MEL score, derived from a commonly used scheme for hormone receptors in breast cancer, may provide a more standardized approach to thresholds using a 0-to-5 scale based on tumor and immune cell PD-L1 staining [54]. Under the MEL criteria, a score of 0 indicates no staining and a score of 1 indicates < 1% staining; both are considered negative. Scores of 2 and above indicate positive staining; 2: ≥1% but < 10%; 3: ≥10% but < 33%; 4: ≥33% but < 66%; and 5: ≥66%. Understanding the caveats associated with PD-L1 testing and creating a uniform testing standard will enhance the utility of PD-L1 testing in the guidance of treatment decision-making. However, at this time, PD-L1 testing alone cannot be used as the sole reason to choose one therapy over another in patients with melanoma.

LAG-3

PD-1 is one of many immune checkpoint molecules that tumor cells may co-opt in order to evade immune attack [59]. Preclinical evidence from mouse models has implicated upregulation of alternative checkpoint molecules as a potential means of resistance to anti–PD-1 therapy [60]. Specifically, the co-expression of lymphocyte-activation gene 3 (LAG-3) with PD-1 has been observed in animal models of melanoma recurrence following adoptive cell transfer. Notably, PD-L1 blockade alone did not provide benefit, but treatment with a combination of anti–PD-L1 and anti–LAG-3 agents resulted in tumor regression when given at the time of recurrence [61]. In humans, expression of LAG-3 within the tumor was associated with response to combination checkpoint inhibition with anti–LAG-3/anti–PD-1 therapy following progression on an anti–PD-1/anti–PD-L1 antibody [62]. Patients who had tumors with ≥1% cells expressing LAG-3 had an overall response rate of 18% vs 5% in patients with < 1% of cells expressing LAG-3; this association was independent of PD-L1 expression [62]. This work provides evidence for the involvement of alternative immune checkpoints in resistance to anti–PD-1 therapy and lays the foundation for the evaluation of combination immunotherapy in patients with metastatic melanoma.

Immune cell infiltration

Because checkpoint inhibitors work by enhancing the antitumor activity to the host immune system, characteristics of the tumor immune microenvironment may provide valuable insight into how robust the response may be following treatment with these agents. Serial biopsies from patients receiving pembrolizumab showed that a greater density of preexisting $CD8^+$ tumor-infiltrating lymphocytes—which are negatively regulated by the PD-1/PD-L1 axis—at the tumor invasive margin was correlated with response to anti–PD-1 therapy, suggesting that release of the negative regulation of these T cells may provide greater antitumor activity [63]. A predictive model was developed based on $CD8^+$ cell density at the tumor invasive margin and this model was tested in 15 patients [63]. The model successfully predicted 4 of 5 patients progressing on treatment and 9 of 9 patients who achieved a response [63]. Although the use of $CD8^+$ cell density at the tumor invasive margin as an independent predictive factor for response to anti–PD-1 therapy holds great promise, further validation in a large

cohort is warranted. The study also demonstrated that release of interferons from $CD8^+$ T cells may drive PD-L1 expression in the tumor (adaptive immune resistance) [63]. Additionally, a more clonal T-cell receptor repertoire at baseline, potentially suggesting a more specific antitumor response, was correlated with response in these patients [63].

Immune effector sensitivity

A positive correlation between an immune-related (interferon [IFN]-γ responsive) gene expression signature and clinical benefit has been reported in patients treated with ipilimumab [64,65]. In light of this and because the nature of the immune microenvironment of a tumor at baseline is associated with efficacy of immune checkpoint inhibition, the assessment of an individual's immune signature to predict treatment outcome continues to be an area of active investigation [66]. This emerging concept, known as immunoprofiling, relies on immunoscore, an assessment of the type, density, and location of immune cells [66,67].

Genomic characterization of the melanoma microenvironment has also provided insight into potential predictive biomarkers for efficacy of newer immune checkpoint inhibitors and combinations, and could contribute to effective immunoprofiling. Analysis of differentially expressed genes at baseline from tumors of patients responding and not responding to anti–PD-1 therapy demonstrated a set of genes, upregulated in nonresponders, linked to mesenchymal and inflammatory tumor phenotypes (termed IPRES [innate anti–PD-1 resistance]) [68]. Conversely, mutations in the DNA repair gene *BRCA2* were enriched in tumors that responded to anti–PD-1 therapy [68].

Predictive value of the host microbiota

Evidence suggests that the gut microbiome can impact the systemic immune response, both quantitatively and qualitatively, and that microbial pathogens drive tumorigenesis in 15–20% of cancer cases [69,70]. The presence of certain gut bacteria, including *Akkermansia muciniphila* and *Bifidobacterium*, was associated with improved efficacy of PD-1 blockade in animal models [71,72]. Indeed, clinical response to immune checkpoint inhibitors has been linked to relative abundance of *A muciniphila* in patients with cancer, and the use of antibiotics either preceding or directly following initiation of immune checkpoint inhibitor therapy was associated with significantly shorter PFS and OS [72]. In melanoma, significant differences have been observed in the composition and diversity of the gut microbiome between responders and nonresponders to anti–PD-1 immunotherapy [73]. These data suggest that profiling the host microbiota may have validity as a predictive biomarker, and that supplementation with "probiotics" may present a treatment opportunity in the future and is emerging as an active area of research [69,71–73].

Individualizing treatment utilizing multiple biomarkers

Because the immune-cancer interaction is based on several distinct parameters, the importance of which is unique to each patient, the cancer immunogram [74] was devised as a means to identify the most prominent biomarkers on an individual patient level (Fig. 2). Encompassing several identified or plausible biomarkers, the immunogram takes into account tumor foreignness (mutational load); general immune status (lymphocyte count); immune cell infiltration (intratumoral T cells); tumor sensitivity to immune effectors (major histocompatibility complex expression, IFN-γ sensitivity); and the absence of checkpoints (PD-L1 expression), soluble inhibitors (interleukin [IL]-6, C-reactive protein), and inhibitory tumor metabolism (LDH, glucose utilization) to guide treatment decisions [74].

Of course, understanding the appropriate treatment strategy aims not only to provide the optimal efficacy for patients with melanoma but also to spare them undue toxicity. By understanding predictive factors for treatment efficacy and toxicity, physicians have the potential to offer a comprehensive patient-specific algorithm for personalized therapy in melanoma. For example, recent evidence suggests that baseline circulating IL-17 significantly correlated ($P = .02$) with the development of grade 3 immune-related diarrhea/colitis in patients treated with ipilimumab [75].

In addition to refinement of pretreatment predictive strategies, efforts are ongoing to better understand tumor biology in patients with an early response to therapy, in order to provide on-treatment biomarkers that can establish, early in treatment, if a therapy is likely to provide benefit.

Clinical rationale for on-treatment biomarkers

Although tremendous strides have been made in the identification of baseline biomarkers predictive of response to therapies in melanoma, there is an ongoing need to refine these strategies that will require considerable effort. Further complicating the situation is that many patients who respond to antimelanoma therapies will eventually develop acquired resistance and ultimately progress. Consistent tumor-level monitoring has the potential to provide early indication of treatment response and resistance, and may help to establish mechanisms of acquired resistance that can provide the basis for rational treatment sequencing.

Liquid biopsy

Liquid biopsies represent a promising, feasible, and noninvasive assessment method that may be used for longitudinal detection and monitoring of melanoma. Blood sampling of on-treatment biomarkers offers the potential for monitoring of treatment response, treatment-associated toxicity, and onset of treatment resistance.

Noninvasive blood biomarker assays allow for regular dynamic monitoring of disease without the need for traditional assessments such as computed tomography scans. With advances in cell-free nucleic acid detection and quantification, circulating tumor DNA (ctDNA) has emerged in recent years as a valid and important surrogate marker of tumor burden and treatment response in multiple cancer types, and melanoma is no exception [76].

Most living cells release nucleic acids, often contained within small vesicles known as exosomes [77,78]. However, the amount of circulating DNA originating from cancer cells can be substantially higher than normal and may be related to disease stage [79] as well as the rate of cancer cell turnover and subsequent DNA release during apoptosis or necrosis [80,81]. Although not yet ready for use in all patients with melanoma, liquid biopsies remain an avid area of biomarker research.

Utility of ctDNA monitoring in patients with metastatic melanoma

Baseline ctDNA levels have been found to be correlated with tumor burden, and therefore high levels of ctDNA may be a reflection of higher tumor load, which is associated with poor prognosis [82,83]. For instance, an analysis of baseline ctDNA demonstrated that patients with detectable levels of *BRAF* V600E had worse OS and PFS in response to dabrafenib or trametinib treatment compared with patients who had undetectable *BRAF* V600E ctDNA [84]. In a separate analysis, lower levels of ctDNA at baseline (*BRAF* or *NRAS*) were significantly associated with response to therapy and PFS regardless of treatment received (BRAF ± MEK inhibitor therapy or immune checkpoint inhibition; Table 1) [85].

The sensitivity of ctDNA analysis for the assessment of tumor burden may also allow for the on-treatment monitoring of response and progression before evidence of clinical progression (Fig. 3). In a longitudinal assessment of patients with *BRAF* V600–mutant metastatic melanoma treated with dabrafenib + trametinib, ctDNA levels declined

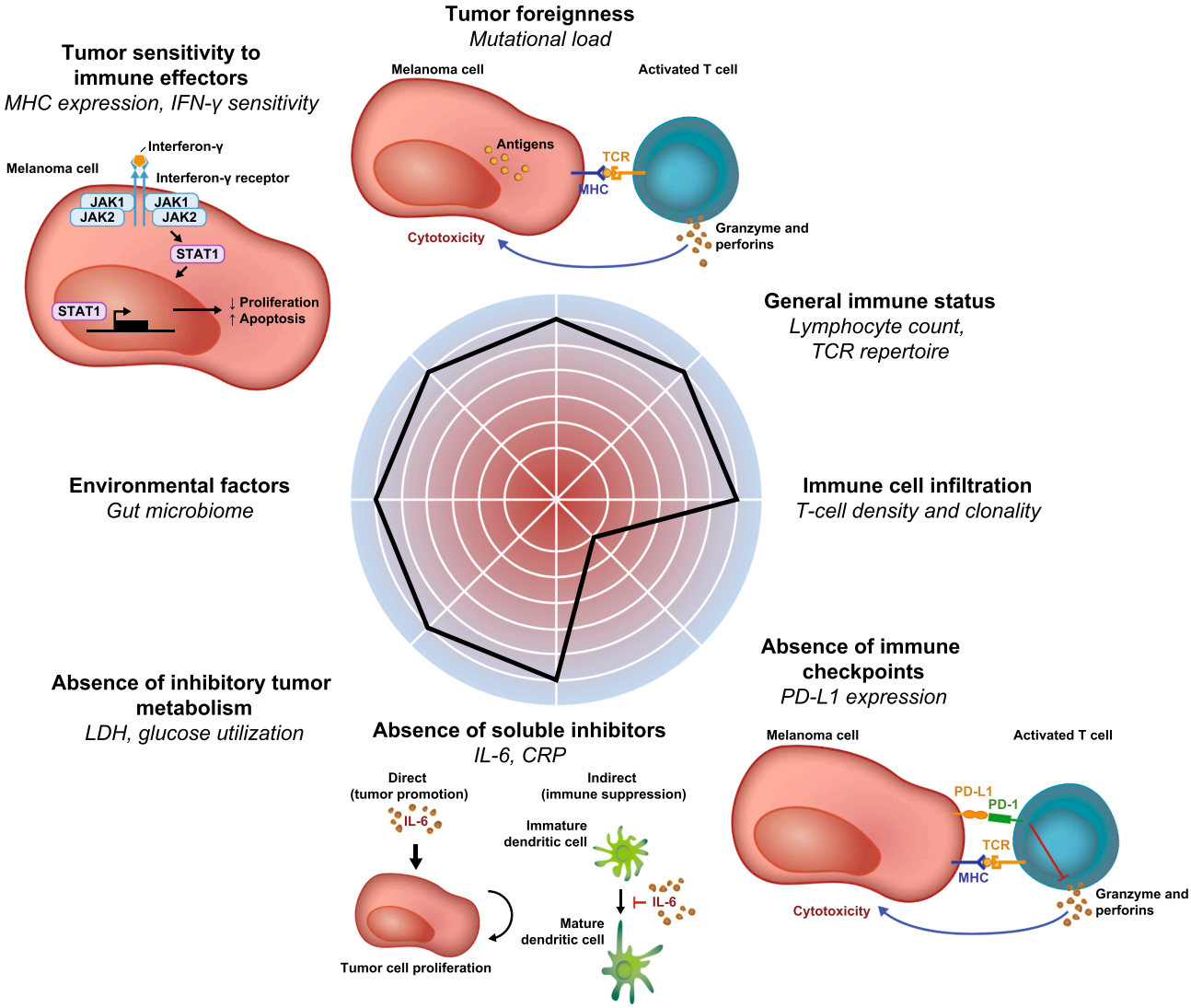

Fig. 2. Inside a modified cancer immunogram. The cancer immunogram is displayed with the proposed addition of environmental factors due to emerging evidence suggesting a predictive role for the gut microbiome. The immunogram depicts favorable (blue) and unfavorable (red) conditions of the cancer-immune interaction across 8 parameters for a hypothetical patient (who may respond well to immune checkpoint inhibition therapy; black line). Inset schematics demonstrate components of the immunogram at the cellular level. CRP, C-reactive protein; IFN, interferon; IL, interleukin; JAK, Janus kinase; LDH, lactate dehydrogenase; MHC, major histocompatibility complex; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; STAT, signal transducers and activators of transcription; TCR, T-cell receptor. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rapidly ($P < .001$) following initiation of treatment and became undetectable in 60% of patients by week 6 [86]. A significant PFS benefit ($P < .001$) was also observed in patients whose *BRAF* V600–mutant ctDNA declined to undetectable levels during treatment vs patients in whom *BRAF* V600–mutant ctDNA remained detectable. Moreover, the increase in *BRAF* V600–mutant ctDNA levels during treatment was always followed by clinical progression within 2 months and preceded clinical progressive disease in 44% of cases [86], which is consistent with prior reports [87]. The authors hypothesized that *BRAF* V600–mutant ctDNA likely represents proliferative tumor burden and not simply tumor mass as detected by computed tomography imaging; therefore, an increase in ctDNA levels could represent an early marker for disease progression (Table 1) [86].

These findings were assessed within a prospective phase 2 trial that evaluated rechallenge with dabrafenib + trametinib in patients who previously progressed on a BRAF inhibitor (with or without a MEK inhibitor) and had discontinued this therapy for at least 12 weeks (Table 1) [88]. Patients were also required to have previously received immune checkpoint inhibitor therapy. The trial reported a tumor response to a combination of dabrafenib + trametinib in 32% (8/25) of patients with advanced *BRAF* V600–mutant melanoma who previously had progression on BRAF ± MEK inhibitors. Notably, patients who achieved a partial response had significantly lower *BRAF* V600–mutant ctDNA levels after 2 weeks of treatment than those with no response ($P = .040$). Conversely, patients with detectable levels of *BRAF* V600–mutant ctDNA after 2 weeks of treatment had a significantly worse PFS (1.8 months [95% CI, 1.2–2.4] vs 5.9 months [95% CI, 4.9–6.9]; $P = .001$). The results from this trial supported the feasibility of rechallenge with mitogen-activated protein kinase (MAPK) pathway inhibitors, which may be a potential important treatment option in patients who have progressed following BRAF ± MEK inhibition and immune checkpoint inhibitor therapy. Also, the early predictive significance of *BRAF*-mutant ctDNA could provide a useful tool in this patient population, due to the higher rate of resistance observed on rechallenge.

Emergent data suggest that the value of ctDNA analysis is not limited to patients with *BRAF* mutations. In a longitudinal study of a wider range of potential biomarkers, on-treatment analysis of ctDNA in

Table 1
Summary of studies evaluating the on-treatment monitoring of ctDNA.

Reference	Patients (n; genotype)	Subgroups	Treatment	Monitoring frequency	Key data
Santiago-Walker et al. [84]	641; *BRAF* V600E 79; *BRAF* V600K	BREAK-2 n = 92 BREAK-3 n = 250 BREAK-MB n = 172 (Group A, n = 89; No prior local brain therapy; Group B, n = 83; Prior local brain therapy) METRIC n = 322	BREAK-2: Dab (n = 92) BREAK-3: Dab (n = 187); DTIC (n = 63) BREAK-MB: Dab (n = 172) METRIC: Tram (n = 214); Chemo (n = 108)	Variable	• *BRAF* V600mut detectable in cfDNA at baseline in 76% and 81% of pts with *BRAF* V600E– and V600K–mutant tumors, respectively • Pts without *BRAF*mut ctDNA had longer PFS and OS • Presence of *BRAF*mut ctDNA was an independent prognostic factor for PFS in 3/4 studies • Pts negative for *BRAF*mut ctDNA had higher response rates to Dab and Tram
Gray et al. [85]	48; *BRAF* V600E/K/R or *NRAS* Q61R/K/L mutation	N/A	MAPK inhibitor (n = 29) Immunotherapy (n = 19)	Variable	• ctDNA 35/48 pts at baseline, lower ctDNA levels at baseline significantly associated with response to treatment and prolonged PFS, irrespective of therapy type • ctDNA decreased significantly in pts treated with MAPK inhibitors in accordance with response • *NRAS*mut ctDNA 3/7 pts progressing on kinase inhibitors
Schreuer et al. [86]	36; unspecified *BRAF* V600mut	First plasma sample: Group 1 before therapy n = 16 Group 2 after therapy n = 20	Dab + Tram (n = 34); Dab (n = 2)	Monthly	• Ongoing response: increase in *BRAF* V600mut ctDNA 0/9 pts • PD: increase in *BRAF* V600mut ctDNA 19/27 pts
Schreuer et al. [88]	23; *BRAF* V600E 2; *BRAF* V600K	N/A	Dab + Tram (n = 25)	Baseline, 2 weeks, 2 months, DP	• PD: detectable *BRAF* V600mut ctDNA 14/18 pts (78%)
Lee et al. [89]	51; *BRAF* V600E/K 35; Non-*BRAF* V600E/K*	Group A n = 36 ND ctDNA at baseline and during therapy Group B n = 22 detectable baseline ctDNA, but ND early in therapy Group C n = 18 ctDNA detectable baseline and during therapy	Single-agent anti–PD-1 (n = 50); Combination anti–PD-1 and anti–CTLA-4 (n = 36)	Every 2 weeks	• RR and PFS significantly better in A + B than C

Chemo, chemotherapy; cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; Dab, dabrafenib; DTIC, dacarbazine; MAPK, mitogen-activated protein kinase; mut, mutation; N/A, not applicable; ND, not detected; OS, overall survival; PD, progressive disease; PD-1, programmed death 1; PFS, progression-free survival; pt, patient; RR, response rate; Tram, trametinib.

* *BRAF* L597Q/L597R/G464E/G466E, *NRAS* Q61H/Q61K/Q61L/Q61R, and *KIT* K642E.

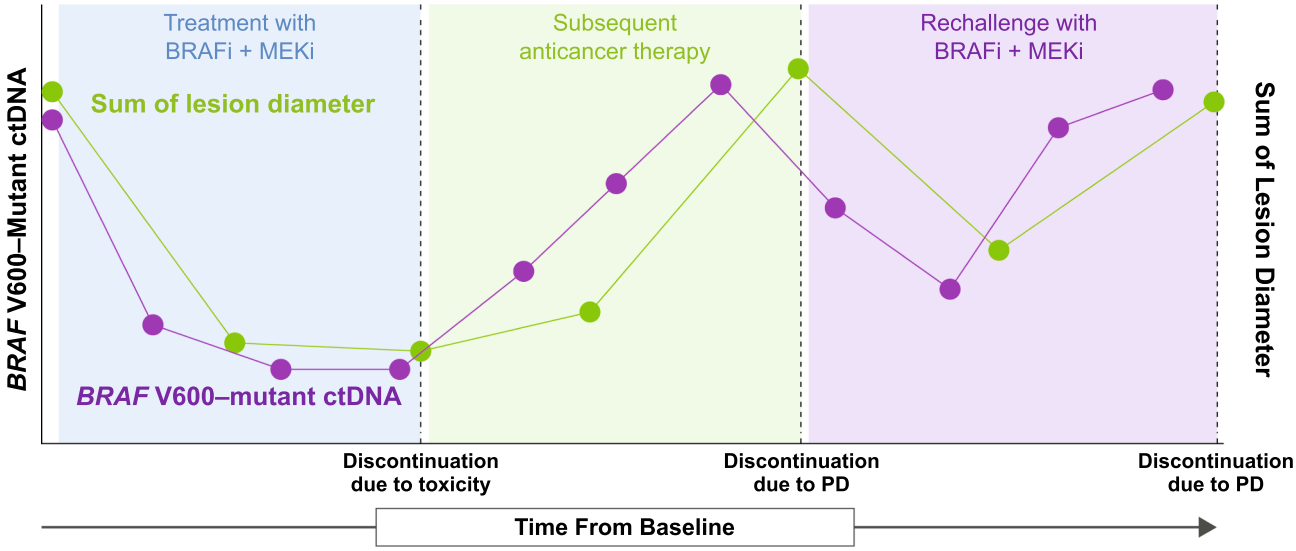

Fig. 3. On-treatment monitoring of ctDNA: hypothetical patient journey. In this hypothetical patient case, a decline in ctDNA following initiation of BRAFi + MEKi treatment is observed prior to radiographic evidence of tumor reduction. Following discontinuation of targeted therapy due to toxicity and initiation of subsequent therapy, an increase in *BRAF*-mutant ctDNA is observed preceding radiographic evidence of progression. Following discontinuation due to progression, the patient receives rechallenge with BRAFi + MEKi therapy, which initially produces a response, initially observed as a reduction in ctDNA, followed by subsequent progression. BRAFi, BRAF inhibitor; ctDNA, circulating tumor DNA; MEKi, MEK inhibitor; PD, progressive disease.

patients who had *BRAF*, *NRAS*, or *KIT* mutations and were treated with anti–PD-1 inhibitors demonstrated similar results, suggesting that the ctDNA profile of patients at week 8 may be a sensitive and reliable marker for treatment outcomes [89]. In another longitudinal study of patients with stage IV melanoma positive for *BRAF* or *NRAS* mutations and treated with anti–PD-1 inhibitors, ctDNA analysis at baseline and within 12-weeks of treatment accurately differentiated pseudoprogression, defined as radiologic progression not confirmed as progressive disease at follow-up assessment, from true disease progression. However, 2 of the 20 patients with confirmed disease progression by radiologic review exemplified a favorable ctDNA profile within 12 weeks of treatment, suggesting ctDNA may be useful in differentiating pseudoprogression in patients treated with checkpoint inhibitors but may be limited in its use as an absolute predictor of response [90].

Although these data are intriguing and could provide clinical utility in the future, it is important to note that trials thus far have included small cohorts, and prospective large-scale analyses evaluating the benefit of monitoring ctDNA in patients with melanoma are necessary. Indeed, pertinent questions remain regarding the utility of switching therapy or adding an additional agent at the first sign of ctDNA increase. Furthermore, in patients with detectable ctDNA levels at baseline that do not decline rapidly, is a change in therapy recommended and if so, when? At present, techniques are variable; for instance, samples may be derived from serum, plasma, or peripheral blood lymphocytes, and several detection methods are available, including polymerase chain reaction (eg, allele specific, droplet digital, and competitive allele specific) and next-generation sequencing with or without whole-exome sequencing [91]. To accompany a consensus guideline on the previous questions, a standardized procedure with an established sensitivity threshold and validated methodology should be agreed on and utilized.

On-treatment monitoring of the immune microenvironment

As our understanding of the immune response following immune checkpoint inhibition has increased, evidence has emerged that specific immune-related changes in the tumor microenvironment following treatment initiation could provide an early indication of outcomes (Table 2).

Initial investigations centered around on-treatment monitoring of response to ipilimumab. As ipilimumab exerts antitumor activity through enhancement of immune response via activation of T-cell proliferation [12], absolute lymphocyte count (ALC) following treatment initiation has garnered interest as a potential biomarker. For example, an increase in ALC from baseline to week 12 was found to be significantly associated with OS and disease control, reflecting information derived from established markers such as LDH [92]. An increase in $CD8^+$ T cells within the tumor has been observed following ipilimumab treatment [93], and increased proliferating $CD8^+$ T cells ($CD8^+/Ki67^+$) within the tumor has been associated with response to ipilimumab therapy [63]. These findings were expanded on to include a greater subset of T-cell markers in a wider range of treatments. Early on-treatment assessment of patients treated with anti–PD-1 agents demonstrated a marked increase in T-cell subsets ($CD8^+$, $CD4^+$, $CD3^+$) and immune checkpoint molecules (PD-1, PD-L1, LAG-3) in patients who responded to therapy vs those without a response [94]. Importantly, the early on-treatment expression of these markers appeared to be a more sensitive indicator of response to therapy than pretreatment values, many of which were not significantly associated with response in this study. Interestingly, in both of these studies, elevated on-treatment granzyme B expression, a marker for active cytolysis, was significantly correlated with response to anti–PD-1 therapy and may represent an additional marker for immune-mediated antimelanoma activity in response to these agents [63,94].

Gene expression profiling has also provided insight into underlying genomic changes following initiation of checkpoint inhibitor therapy and potential association with response. In a study evaluating neoadjuvant ipilimumab therapy, a proinflammatory gene signature marked by increased expression of IFN-γ–inducible genes and Th1-associated markers was observed 6 weeks after treatment initiation [65]. The identified gene set was significantly associated with clinical outcomes, including relapse-free survival ($P = .034$), implicating checkpoint inhibitor–mediated induction of inflammatory response in the antitumor activity of these agents.

For many years, patients with melanoma at high risk of relapse following resection have derived clinical benefit from adjuvant therapy with IFN-alfa-2b [95]. Evidence from patients with resected melanoma treated with IFN-alfa-2b suggests that therapeutically induced

Table 2
Summary of studies evaluating on-treatment monitoring on the immune microenvironment.

Reference	Patients (n)	Treatment	Monitoring interval	Markers assessed	Key data
Simeone et al. [92]	95	Ipi	Baseline, wk 4, 7, 10, 12	LDH; CRP; Tregs; WBC; ALC	• Disease control and survival were significantly associated with decreasing levels of LDH, CRP, and FoxP3/Tregs, and increasing ALC, between baseline and wk 12
Tarhini et al. [93]	33	Ipi (neoadjuvant)	Baseline and wk 6	Multicolor flow cytometry and IHC	• Decrease in circulating Lin1−/HLA-DR−/CD33$^+$/CD11b$^+$ MDSC (P = .03) and increase in circulating Tregs (P = .034) from baseline and wk 6 significantly associated with improved PFS • Intratumoral CD8$^+$ T cells were significantly increased following Ipi (P = .02) and changes in intratumoral Tregs and Lin1−/HLA-DR−/CD33$^+$/CD11b$^+$ MDSC inversely correlated with clinical benefit
Tarhini et al. [65]	27	Ipi (neoadjuvant)	Baseline and wk 6	mRNA expression profiling	• A gene signature corresponding to an immune active/proinflammatory tumor microenvironment was associated with relapse-free survival and OS following 6 wk on Ipi therapy • Following Ipi treatment, an increase was observed in IFN-γ–inducible genes and Th1-associated markers
Tumeh et al. [63]	46	Pembrolizumab	Baseline and ≥1 biopsy at 20–60 days, 60–120 days, > 120 days after treatment start	Multimarker IHC panel	• Responders showed proliferation of intratumoral CD8$^+$ T cells that directly correlated with radiographic reduction in tumor size • Pretreatment samples from responders showed higher numbers of CD8$^+$, PD-1$^+$, and PD-L1$^+$ expressing cells at the invasive tumor margin and inside tumors
Chen et al. [94]	53	Anti–CTLA-4 (n = 53) then anti–PD-1 (n = 46) at progression	Baseline then multiple time points	12-marker IHC panel and NanoString analysis	• Adaptive immune signature in on-treatment biopsies of PD-1 responders (n = 5) distinct from nonresponders (n = 6) • Highly significant difference in the expression of markers for T-cell subsets (CD8, CD4, and CD3), immunomodulatory receptors (PD-1, PD-L1, and LAG-3), and higher activation status (CD45RO, FOXP3, granzyme B, and CD57) in the early on-treatment tumor samples of PD-1 responders compared with nonresponders

ALC, absolute lymphocyte count; CRP, C-reactive protein; CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; HLA-DR, human leukocyte antigen–antigen D related; IFN, interferon; IHC, immunohistochemistry; Ipi, ipilimumab; LAG-3, lymphocyte-activation gene 3; LDH, lactate dehydrogenase; MDSC, myeloid-derived suppressor cell; OS, overall survival; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; Th, helper T cell; Treg, regulatory T cell; WBC, white blood cell.

autoimmunity has the potential to be a surrogate predictive biomarker [96]. Recent studies have identified several proinflammatory serum markers such as IL-2Rα, IL-12p40, and IFN-α that are associated with relapse-free survival in patients receiving interferon [97]. The evaluation of blood-based proinflammatory markers could potentially be useful in the metastatic setting as well, although further evaluation is warranted.

These data demonstrate that an understanding of immune response and assessment of early on-treatment immune-mediated changes in the tumor and surrounding microenvironment may hold great promise. However, similar to ctDNA profiling, evaluation of these tools remains in its infancy, and further validation and consensus guidelines will be required before widespread clinical adoption.

Conclusion

The validation of baseline and on-treatment biomarkers for response to available melanoma therapies has the potential to move treatment toward a more personalized therapeutic approach. In the not-too-distant future, the optimal treatment could be preselected based on pretreatment assessment, and/or timely sequencing of therapy could be provided based on early on-treatment markers predictive of the likelihood of long-term response. Research in biomarkers is a rapidly evolving field that is destined to change the way we care for melanoma patients in the near future.

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Contributions

All authors contributed to the conception, drafting, and critical review of the manuscript and provided final approval. A Tarhini designed research, performed research, contributed vital new reagents or analytical tools, collected data, and analysed/interpreted data.

Conflict of interest disclosure

A Tarhini served as a consultant for Novartis, Bristol-Myers Squibb, Merck, Incyte, and NewLink Genetics. R Kudchadkar served as a consultant for Bristol-Myers Squibb and Array, and received research funding from Bristol-Myers Squibb and Merck. All authors acknowledge nonfinancial support from ArticulateScience LLC.

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