Biomarkers in melanoma: where are we now?

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Practice points

- Biomarkers are playing an expanding role in the management of melanoma.
- In local/regional disease, the primary predictors of prognosis and recurrence are local features, including depth of invasion, ulceration and extent of lymph node involvement.
- In advanced melanoma, several markers have clearly defined prognostic (lactate dehydrogenase) or therapeutic implications (*BRAF* or *CKIT* mutational status).
- Novel, promising biomarkers with implications for immune-based therapy include the tumor expression of PD-L1, changes in the absolute lymphocyte count and immune-related gene-expression panels.
- Blood-based biomarkers, including circulating DNA and circulating tumor cells, are promising for monitoring recurrence and therapy response.

SUMMARY Melanoma has traditionally been associated with limited treatment options, and as such, biomarkers such as histopathologic staging and serum lactate dehydrogenase focused on prognosis. The development of effective treatment options shifted the search to biomarkers for predicting response and resistance to therapy, an arguably more critical goal. Specific genetic alterations (e.g., *BRAF*^{V600} and *KIT* mutations) predict response to molecularly targeted agents and are routinely used in clinical practice. Other promising biomarkers include T-cell characteristics (the circulating and tumor microenvironment), tumor expression of PD-L1, circulating DNA, circulating tumor cells and miRNAs. In this article, we discuss the status of the currently used and experimental tumor- and blood-based biomarkers for melanoma prognosis and response to targeted and immune therapies.

Melanoma is an aggressive cutaneous malignancy that caused nearly 10,000 deaths in the USA in 2013 [1]. The incidence of melanoma has been increasing in recent decades, probably due to epidemiologic factors, particularly ultraviolet light exposure [2,3]. The clinical presentation of this disease ranges from minimally invasive localized involvement to disseminated metastases. Accordingly, the clinical outcome varies widely from a >95% cure rate with surgical excision of early-stage melanoma to a historically dismal median survival of less than 9 months for patients with metastatic disease [4,5]. The recent development of small-molecule inhibitors targeting oncogenic drivers of melanoma, as well as immune-based therapies, have dramatically improved treatment options and patient outcomes in many cases.

Identifying predictive biomarkers in order to inform melanoma treatment decisions and prognosis has been a major effort over the last several decades and has gained importance following

KEYWORDS

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the availability of newer, effective therapeutics. Traditionally, histopathologic characteristics of the tumor were the main informants of prognosis, particularly depth of invasion, ulceration and presence of lymph node or distant metastases [4]. Although these markers provide essential prognostic information, they have limited applications for treatment decisions. Furthermore, marked heterogeneity in outcomes is noted even among patients with similar clinical presentations and histopathologic markers. More recently, both tumor- and blood-based genetic and immune biomarkers have been identified with additional critical prognostic and therapeutic implications. These markers are already being used in order to guide treatment decisions for several of the newly developed therapies, although better predictors are needed. Ongoing efforts have focused on predictors of response to immune-based therapies, markers of resistance to molecularly targeted therapies and prognostic biomarkers. In this article, we will review the biomarkers that are currently incorporated into clinical practice and discuss other promising candidates.

Tissue-based biomarkers

• Traditional pathologic biomarkers

Melanoma has traditionally been classified based on the pathological characteristics of the primary tumor and the extent of regional or distant metastases. A number of pathological factors following surgical and/or radiographic staging inform prognosis. Tumor thickness, ulceration, number of mitoses (only for thin melanomas), lymph node involvement and distant metastases are incorporated into the American Joint Committee on Cancer (AJCC) melanoma staging system from a multivariate analysis of >38,000 patients [4]. The tumor thickness and depth of invasion (AJCC ‘T’ stage) of localized disease strongly influence risk of recurrence; melanomas with <1 mm invasion (AJCC T1) had >95% long-term survival compared with approximately 50% for tumors >4 mm thick (T4) [4,6]. Tumor ulceration correlates with inferior prognosis in conjunction with depth of invasion; for example, in patients with primary tumors that are >4 mm thick, the presence of ulceration worsens long-term survival (71 vs 53%). Lymph node involvement (AJCC ‘N’ stage) also plays a role in the risk of recurrence. Among patients with a single focus of microscopic lymph node involvement (AJCC TxN1a), extended survival occurs in 70%, compared

with only 39% among those with four or more lymph nodes involved (TxN2b). In the setting of metastatic disease, the extent of organ involvement (AJCC ‘M’ stage) influences outcomes; cutaneous-only versus lung-only versus visceral involvement (AJCC M1a, M1b or M1c, respectively) have historical 1-year survival rates of 62, 53 and 33%, respectively. Elevated blood lactate dehydrogenase (LDH) classifies patients in the M1c group as well, due to its correlation with an adverse outcome.

These traditional markers also have limited therapeutic implications. For example, patients with AJCC stage IIb, IIc and III (deep ulcerated tumors or presence of lymph nodes) have modest improvements in progression-free survival (PFS) and possibly overall survival (OS) when treated with high-dose IFN- α [7–9] following disease resection. In addition, patients with stage IVc melanoma have inferior outcomes when treated with high-dose IL-2 or ipilimumab compared with those with stage IVa or IVb disease [10,11]. Although these traditional markers have important prognostic value, their limited therapeutic implications and lack of predictive precision makes the identification of additional biomarkers a critical, unmet need.

• Targeted therapy biomarkers

The identification of recurrent oncogenic ‘driver’ mutations has transformed melanoma therapeutics over the past few years. *BRAF*^{V600} mutations are the most frequently identified recurrent alterations and occur in nearly 50% of patients [12,13]. These mutations confer sensitivity to approved small-molecule inhibitors of BRAF (vemurafenib and dabrafenib) and MEK (trametinib), which improve clinical outcomes compared with cytotoxic chemotherapy [14–16]. Most patients experience tumor regression but ultimately experience disease progression at a median of approximately 6 months; a subset, however, either are primarily refractory to BRAF inhibitors or, conversely, experience prolonged responses. Intensive efforts to identify predictive markers for these patients are ongoing; primary resistance mechanisms probably include mutations that activate parallel cell signaling pathways [17,18]. Mutations in *CKIT* occur in only 1–2% of cutaneous melanomas, but in 15–20% of melanomas arising from acral or mucosal surfaces [19,20]. *KIT* mutations, particularly the most common *KIT*^{L576P} and *KIT*^{K642E} mutations, confer sensitivity to imatinib and other tyrosine kinase inhibitors

(sunitinib and dasatinib) in many cases [21–23]. *NRAS*-mutant melanoma is the second most common oncogene-defined cohort (15–20% of melanomas) and lacks approved molecularly targeted agents, although MEK inhibitor therapy as a single agent (binimetinib [MEK162]) and in combination with other small-molecule inhibitors appears promising [24–26]. Furthermore, pre-clinical data suggest that loss of *CDKN2A* may predict benefit from inhibitors of CDK4/6 (i.e., LEE011 and palbociclib), which are agents that may have real promise in melanoma [27]. A wide range of other mutations have been identified in melanoma that may serve as biomarkers for experimental therapies (e.g., *PTEN*, *NF1*, *BRAF* fusions and atypical *BRAF* mutations) [28–31]. Tumor heterogeneity and mutations acquired during therapy remain challenges to reliable genetic biomarkers [32].

From a prognostic standpoint, these recurrent oncogenic alterations may have some importance. Unselected patients with *NRAS* mutations appear to have inferior OS compared with other genetic cohorts (*BRAF* mutant, *NRAS/BRAF* wild-type), although this has not been confirmed in every study [33–35]. The impact of *BRAF* mutations on prognosis is less clear since an effective targeted therapy has now been identified that improves OS. Other genetic changes that have been linked to a poor prognosis include the amplification of chromosomes 8q24 or 11q13 (which contain *MYC* and *CCND1*, respectively) [36].

• Immune-based therapy biomarkers

In contrast to the molecularly targeted agents, efforts to conclusively identify predictive biomarkers for the immune-based therapies have been largely unsuccessful to date, although a number of promising candidates have been identified. Efforts have largely focused on genetic changes and protein expression in the tumor itself, characteristics of the immune infiltrate (reviewed in this section), circulating immune cells and host genetic factors (reviewed in the following section). Most investigational biomarkers represent distinct but complimentary facets of an effective immune response. For example, tumors that are destined to respond to a particular immune therapy could potentially express or produce molecules that induce specific changes in the immune infiltrate and/or circulating lymphocytes.

Genetic changes and gene expression in the melanoma may influence the immune

response by inducing antigen expression or cytokine production [37–39]. Gene-expression signatures have shown promise in predicting benefits from the immune-based therapies. An expression panel consisting of six chemokines correlated chemokine expression in melanoma clinical specimens with CD8⁺ T-cell infiltration, suggesting that tumor production of chemokines/cytokines may be necessary for T-cell recruitment and effective immune responses [40]. A retrospective cohort study of 28 melanoma patients treated with IL-2 provides clinical evidence for this hypothesis; tumors expressing immune/inflammatory genes in a separate gene-expression panel had much higher rates of objective response compared with those overexpressing melanocyte-lineage genes (86 vs 33%) [41]. Based on these results, an observational prospective trial has been performed in melanoma patients treated with IL-2 (SELECT trial) and its results should be available later this year. A distinct signature, primarily consisting of immune-related genes, including chemokines, appeared to correlate with improved survival from the MAGE-A3 fusion protein immunotherapy (hazard ratio [HR] for OS: 0.37; *p* = 0.06) [42]. This signature also showed a trend towards improved survival in an independent cohort of non-small-cell lung cancer patients receiving the therapy in the adjuvant setting (HR: 0.42; *p* = 0.06). Gene signatures may also have value for the new immune checkpoint inhibitors. In a Phase II trial of patients treated with ipilimumab, an immune-overexpressing gene profile (IFN- γ -inducible genes and Th1-associated markers) with low expression of melanocyte-related genes correlated with improved outcomes [43].

The effects of oncogenic driver mutations on response to immune-based therapy have been less well characterized. *NRAS* mutations, which predict poor outcomes in an unselected population, appear to be associated with improved clinical outcomes for those treated with immune-based therapy [33]. In a cohort treated with high-dose IL-2, higher response rates and a trend towards improved PFS and OS were observed for the *NRAS*-mutant group [11]. In a larger study, we also noted that response rates were higher for those with *NRAS*-mutant melanoma compared with *NRAS/BRAF* wild-type melanoma; this effect was particularly notable for newer immune checkpoint inhibitors [44]. This finding needs prospective validation. *BRAF* mutations,

by contrast, appear to have less of an impact on immune therapy outcomes, with equivalent response rates to ipilimumab in a small retrospective study regardless of *BRAF* mutational status [45].

An extremely promising class of immune-based therapies currently being evaluated in clinical trials are agents targeting PD-1/PD-L1 (including nivolumab [BMS-936558], MK-3475 and MPDL3280A). These agents induce objective response rates in 25–45% of patients in early trials, many of which are durable [46–49]. Tumor expression of PD-L1 appears to correlate with benefits on these therapies. In the original Phase I trial of nivolumab, PD-L1 expression seemed to be essential for response; however, subsequent studies have demonstrated that PD-L1-negative tumors can still respond, although with lower frequency than in PD-L1-positive tumors [50,51]. Technical issues regarding the assays and the temporal/spatial heterogeneity of PD-L1 staining may limit its value for determining treatment decisions [52]. In addition, the expression of PD-L1 did not appear to predict benefit from ipilimumab and nivolumab combination therapy in the Phase I trial of this regimen, although this observation may be related to the potency of this combination compared with single-agent nivolumab and/or the influence of ipilimumab on PD-L1 expression [53]. Despite these limitations, PD-L1 does appear to have predictive value and will likely be incorporated into clinical practice, potentially in combination with other markers.

Characterizing the immune infiltrate has also led to additional insights and biomarker candidates. A Phase II trial of patients treated with ipilimumab showed that an increase in the total number of tumor-infiltrating lymphocytes (TILs) between baseline and at 3 weeks on therapy correlated with improved responses [54]. This trial also suggested that baseline expression of FoxP3 and indoleamine 2,3-dioxygenase (IDO) on TILs also predicted improved outcomes, which is a counterintuitive result since these markers have classically been thought to have an immune-suppressive role. Later reports have subsequently suggested that both FoxP3 and IDO are negative prognostic factors in unselected patients and that IDO may even mediate resistance to ipilimumab; therefore, the role of these markers remains unclear [55–57]. Increased tumor infiltration of myeloid-derived suppressor cells has also been linked to inferior prognosis

for patients treated with ipilimumab [58]. In addition, a number of other surface markers on infiltrating T cells may play a role in terms of characterizing the spectrum and types of TILs present in responding patients compared with nonresponders. These include T-cell coactivators (CD27, 4–1BB and CD40L) and checkpoints associated with T-cell exhaustion/repression (CTLA-4, BTLA, PD-1 and TIM-3) [59].

Techniques in order to assess the entire breadth of T-cell infiltration using next-generation sequencing of T-cell receptors may also yield valuable insights [60–62]. This approach characterizes both the overall spectrum of T-cell diversity and clonal expansion by specific T cells. This approach has the potential to investigate relevant populations of T cells and individually expanded clones, providing insights into the immune response. This approach has been tested in the peripheral blood of patients treated with tremelimumab (anti-CTLA4). Although T-cell diversity did not correlate with clinical outcomes, the maintenance of pre-existing, high-frequency clones was associated with improved survival [63,64]. Ongoing studies are characterizing T-cell diversity in the tumor for patients treated with immune checkpoint inhibitors.

• Other tissue-based biomarkers

Melanogenesis is another critical feature of this disease, although the utility of melanin pigment or other intermediates as predictive biomarkers has not yet been well defined. Although a full review of these topics is outside the scope of this article, melanogenesis generates reactive oxygen species, hormones and other intermediates that act as immunosuppressants in the tumor microenvironment [32,65,66]. In a small, unselected group of patients, increased pigmentation in primary tumors and lymph node metastases in patients with metastatic disease was associated with an inferior OS [67]. Investigating the effects of tumor pigmentation on response to immune checkpoint inhibitors would be an intriguing next step.

The role of miRNAs in melanoma tumor progression, cell cycle dysregulation and immune evasion has been increasingly appreciated in recent years and may provide additional candidates for prognostic and therapeutic biomarkers [68]. miR-211 is the miRNA that is most differentially expressed in normal melanocytes and patient tumor samples and appears to function as a melanoma tumor suppressor [69–71].

Melanomas with low levels of miR-211 have high invasive potential, whereas those with high levels have limited invasiveness [69,72,73]. miR-196a and miR-193b also appear to function as melanoma tumor suppressors [74,75]. Specific miRNAs may modulate the immune response as well. miR-210 is upregulated by hypoxia and limits cell lysis by cytotoxic T lymphocytes in preclinical models [76]. miR-34a/c appears to control the expression of ULBP2 [77], a ligand for the natural killer cell immunoreceptor NKG2D and itself a potential marker of poor prognosis, thereby modulating the innate antimelanoma immune response [77,78]. Finally, miR-30 induces the production of the immunosuppressant cytokine IL-10 and reduces immune cell recruitment, potentially explaining its association with poor outcomes [79]. Profiling of miRNAs has not been incorporated into clinical practice at this time. **Table 1** summarizes available and experimental tissue-based and blood-based biomarkers and their current use.

Blood-based biomarkers¹²³

• Traditional blood-based biomarkers

The search for clinically useful blood-based biomarkers has been challenging. To date, only serum LDH has routinely been incorporated

into clinical practice, as an elevated LDH has been repeatedly validated as a poor prognostic factor [80–84]. For example, the current National Comprehensive Cancer Network (NCCN) guidelines recommend checking LDH at the time of stage IV diagnosis and confirming an elevated value with repeat testing [80]. In addition, serum LDH testing is typically performed prior to the commencement of therapy in a clinical trial, as it has been consistently determined to be associated with poorer outcomes of targeted and immune-based therapies and is often used as a stratification factor in randomized trials [10,11,14,85–87].

In nonmalignant settings, LDH is released under circumstances such as nutrient stress (hypoxia in the setting of infarction), necrosis (e.g., hepatic toxicity), infection (e.g., pneumocystis) and hemolysis. In melanoma, the proposed mechanisms of LDH elevation is related to rapid proliferation that leads to a hypoxic tumor microenvironment. Thus, LDH may be thought of a surrogate of tumor hypoxia, and the impact of an elevated LDH on prognosis may be more related to the effects of this tumor-specific hypoxia as opposed to simply being related to tumor burden. Interestingly, patients with an elevated LDH appear to have better outcomes

Table 1. Melanoma biomarkers.

Biomarker	Indication	Ref.
Currently in use in clinical practice		
AJCC staging	Prognosis and need for adjuvant interferon	[4,7]
LDH	Prognosis	[4]
BRAF mutation	Sensitivity to BRAF or MEK inhibitors	[14,16]
KIT mutation	Sensitivity to imatinib (primarily exon 11)	[21]
S-100B [†]	Prognosis	[39,94]
Absolute lymphocyte count	Response to ipilimumab	[113]
Currently used for clinical trial inclusion		
NRAS mutation	MEK inhibitor monotherapy and combination trials	[25,26]
BRAF non-V600 mutations	MEK inhibitor trials	[31,123]
PD-L1 staining	Anti-PD-1/PD-L1 trials	[50]
Gene-expression profiles	Response to IL-2	[41]
Preclinical or clinical support		
CDKN2A loss and CCND1 amplification	Response to CDK4/6 inhibitors	[27]
miRNA	Prognosis (miR-211, miR-196a and miR-193b)	[69,73]
	Immune evasion (miR-210 and miR-34a/c)	[76,78]
Pigmentation	Prognosis and possibly immune evasion	[67]
TIL characterization (IDO, FoxP3 and MDSCs)	Response to immune therapy	[54,58]
T-cell receptor diversity	Response to immune therapy	[64]

[†]Routinely used in Europe, but not widely used in the USA.

AJCC: American Joint Committee on Cancer; IDO: Indoleamine 2,3-dioxygenase; LDH: Lactate dehydrogenase; MDSC: Myeloid-derived suppressor cell; TIL: Tumor-infiltrating lymphocyte.

with antiangiogenic therapy. This statement is based on a subset analysis (elevated LDH) of a randomized Phase II study of carboplatin/paclitaxel (CP) with or without the anti-VEGF monoclonal antibody bevacizumab revealed that patients treated with CP plus bevacizumab had a significantly improved PFS (HR: 0.62) and OS (HR: 0.52) than those treated with CP alone; no differences in PFS or OS were seen in patients with a normal LDH [88]. Confirmation of this finding in a randomized trial specifically enrolling patients with an elevated LDH has not been carried out.

Not surprisingly, an elevated LDH has been associated with poorer outcomes on both high-dose IL-2 and ipilimumab [87,89,90]. While these data are not surprising, given the association of an elevated LDH with poor prognosis, the nearly complete lack of long-term efficacy of both agents in patients with elevated LDH is profound. In a retrospective review of consecutively treated patients at two institutions, only two out of 31 patients (6%) with an elevated LDH had a response to therapy, compared with 21% of the 164 patients who did not have an elevated LDH; in addition, and as expected, the patients with an elevated LDH had poorer survival than those with a normal LDH (median OS: 6.7 vs 23.6 months, respectively) [90]. A similar study was performed by culling data from the Dutch and British compassionate access experience with ipilimumab [87]. In over 200 patients, 73 had elevated LDH. Of these patients, there were seven responders (9.5%) compared with a response rate of 23% in the patients with normal LDH. Furthermore, when a LDH of two or more-times the upper limit of normal (ULN) was used as the cut-off, the 1- and 2-year survival rates were 4.8 and 0%, respectively, for those with an LDH of two or more-times the ULN compared with 51.2 and 34.6%, respectively, in those with an LDH of less than two-times the ULN. It is uncertain whether treatment with anti-PD-1/PD-L1 antibodies will show a similar degree of ineffectiveness in patients with an elevated LDH or whether anti-VEGF agents in combination with immune checkpoint inhibitors will help to overcome the ineptness of IL-2 or ipilimumab treatment in these patients, although two randomized trials ought to help settle this issue (NCT1258855 and NCT01950390).

The S-100 calcium binding protein B (S-100B) is a protein that is expressed on melanocytes and

melanomas that can be detected and quantified in the blood of patients with melanoma [91]. Similarly to LDH, S-100B levels offer prognostic information, presumably in all stages of melanoma, but may be more prognostic than LDH in the stage I–III patient population (but not in the stage IV setting) [92–94]. Nevertheless, S-100B use has not been adopted as part of routine clinical care in the USA, although it is widely used in some European countries [95,96]. Despite the prognostic value of S-100B and LDH, neither a surveillance test, such as prostate-specific antigen (PSA) for prostate cancer, nor a ‘tumor marker’ that can be reliably followed for patients with melanoma and on therapy, akin to carcinoembryonic antigen (CEA), CA19.9 and CA-125, have been identified.

The most promising source of assayable circulating tumor factors will probably not be a protein that is variable expressed on melanoma (S-100B) or a surrogate for tumor cell turnover (LDH), but rather an essential component of the tumor, such as DNA and RNA from circulating tumor cells (CTCs) or cell-free circulating factors, such as DNA, RNA or tumor-secreting exosomes containing nucleic acids. The first attempt to identify an assay that monitors CTC RNA is a four-mRNA reverse transcriptase quantitative PCR (RT-qPCR) assay (MART-1, GalNAc-T, PAX-3 and MAGE-A3) that was detected in an increasing percentage of patients with increasing AJCC stage (15, 30, 75 and 85% for stages I–IV, respectively) [97,98]. Since its initial description, this multimarker assay has been tested in the several clinical settings. For example, in a trial of patients with stage IV melanoma treated with biochemotherapy, mRNA RT-qPCR status after two cycles was predictive of disease progression and OS [99]. Similarly, pretreatment and serial mRNA status (≥ 1 marker being positive) was associated with disease-free survival and OS in patients with resected stage IV melanoma treated with adjuvant immunotherapy [100]. Lastly, in a randomized trial of two adjuvant immunotherapy regimens (melanoma vaccine plus bacillus Calmette-Guérin vs placebo plus bacillus Calmette-Guérin), postsurgery and pretreatment mRNA status (≥ 2 markers being positive) was associated with worse disease-free survival and OS [101].

The isolation and quantification of melanoma CTCs, as opposed to indirect markers such as RT-qPCR of CTC DNA, has been more challenging than in other epithelial malignancies due

to the inconsistent expression in melanoma of epithelial cellular adhesion molecule, the target of the most widely used CTC capture antibody. Nearly a decade ago, a meta-analysis was performed showing that CTC detection was associated with increased risk of death (HR: 2.42; 95% CI: 1.7–3.45) and progression (HR: 3.02; 95% CI: 1.92–4.75) [102]. However, over the past year, a number of groups have developed techniques to more efficiently isolate CTCs from patients with melanoma. These include negative selection strategies, multiple capture antibodies (a 12-antibody set and triple antiganglioside antibodies) and microfluidic chambers [103–106]. It is too early to determine the true clinical utility of these strategies, although there is great promise that these assays may be useful for determining prognosis, for serial monitoring in order to predict relapse or progression and to allow for more extensive molecular analysis.

• Targeted therapy biomarkers

With the development of effective targeted therapies for molecularly defined subsets of melanoma and the improvement of mutational analysis technology, it is now a matter of time until blood-based assays become widely used in order to serially monitor stage IV melanoma patients' disease status during treatment with targeted therapy and possibly to replace tissue-based mutational analysis altogether. In particular, utilizing a number of techniques, including real-time PCR, mass spectrometry, allelic specific PCR, Improved and Complete Enrichment CO-amplification at Lower Denaturation temperature (ICE-COLD) PCR, PCR using locked oligonucleotides in order to suppress wild-type sequences and direct sequencing of RNA or DNA in order to preferentially distinguish the V600E mutant from wild-type *BRAF*, as well as a combination of emulsion-based digital PCR and flow cytometry (so-called 'beads, emulsion, amplification and magnetics, or BEAMing) and the detection and quantification of oncogenic mutations in the plasma, serum, lymphocytes (where CTCs spin down), CTCs and exosomes (secreted membrane-bound vesicles containing tumor nucleic acid) have been well described for mutations in *PI3K*, *KRAS*, *EGFR* and *BRAF* [107–110]. For *BRAF*-mutant melanoma, it is probable that one or more of these techniques will emerge as a reliable diagnostic assay, obviating the need for tissue testing, which can be time consuming (particularly if additional biopsies

are required in order to obtain tissue for such testing). One of these techniques has been tested in over 100 patients with stage II–IV melanoma and appears to have excellent sensitivity and specificity and is quantifiable, allowing for serial monitoring of blood *BRAF* levels in patients treated with *BRAF* inhibitor therapy [111]. It is the serial monitoring of disease relapse (stage I–III) or progression (stage IV) that would truly transform the care of melanoma patients. Furthermore, in the relatively near future, the characterization of resistance mechanisms in the blood of patients on *BRAF*-directed therapy (or any targeted therapy) is expected such that next-line therapies may be selected based on the results of blood analysis.

• Immune-based therapy biomarkers

The most profound breakthrough over the past decade in cancer generally and melanoma specifically is the clinical development of the immune checkpoint inhibitors. Nevertheless, even with single-agent anti-PD-1 therapy or combination ipilimumab plus anti-PD-1 therapy and their relative response rates of 30–50% and 45–50%, respectively, the majority of patients with melanoma will not achieve durable benefits from these therapies [47,50,112]. While there are no routinely tested blood-based biomarkers that guide providers as to who should or should not be offered these therapies, there are emerging data regarding a routinely performed test – the absolute lymphocyte count (ALC) – which may offer valuable information about who is likely to respond to therapy. The original description of this finding came from a retrospective review of patients treated with ipilimumab as part of a compassionate access protocol at the Memorial Sloan-Kettering Cancer Center [113]. Specifically, patients with an ALC of 1000 cells/ μ l or greater were more likely to have a clinical benefit (51 vs 0%) and be alive (median OS: 11.9 vs 1.4 months) after two cycles of therapy than those patients with an ALC of <1000/ μ l after two cycles of therapy. The value of ALC at baseline and serially in patients treated with ipilimumab has been corroborated in other compassionate access protocols and other series and appears to be a valid marker for predicting outcomes of patients with melanoma treated with ipilimumab [87,114–116]. How this will be incorporated into clinical trials or clinical practice remains to be seen. In addition, whether ALC values will be useful with other types of

immunotherapy, such as anti-PD-1/PD-L1 antibodies or combinations of immune checkpoint inhibitors or immune checkpoint inhibitors with targeted therapy, is also unknown.

• Other blood-based biomarkers

As is true of tissue-based analysis, the isolation and evaluation of miRNAs is feasible and, based on early data, offer great potential as blood-based biomarkers in a number of clinical settings [117]. For example, miR-221 was significantly higher in 94 patients with melanoma than in 20 normal controls, was reduced following surgical resection of the melanoma and was increased in some patients at the time of recurrence [118]. An independent analysis that screened 355 miRNAs in the sera of patients with stage I–III melanoma identified a five-miRNA signature that predicted relapse and demonstrated that both miR-221 and miR-103 are increased at the time of recurrence [119]. In addition, a number of other groups are investigating the utility of serum miRNA analysis as a measure of tumor burden, disease recurrence and pharmacodynamic markers in the setting of therapy [120–122]. Nevertheless, much work needs to be done in order to validate the previously described findings and to investigate the predictive utility of markers in the setting of the newer, more effective therapies for melanoma, such as BRAF-targeted therapies or anti-PD-1/PD-L1 antibodies.

Conclusion

Tissue and blood based prognostic biomarkers have been available for many years and continue to play a critical role in the treatment of patients with melanoma. As more effective therapies for melanoma have entered the clinic, however, it is more important than ever to have biomarkers that help guide treatment selection for our patients. One of the great unmet needs

currently for patients with metastatic and high-risk melanoma is the absence of such biomarkers, with the obvious exception of *BRAF* mutational testing. Still, an amazing amount of energy is being focused on the development of predictive blood and tissue biomarkers that will hopefully change the way we approach treatment selection for our patients. Based on the inroads made over the past decade, the future looks bright.

Future perspective

Biomarkers in melanoma have critical implications for prognosis and therapeutic decision-making. Well-established biomarkers used in clinical practice include histopathologic characteristics of the primary melanoma and mutational status in metastatic disease. Over the next several years, it is expected that other blood and tissue biomarkers will be incorporated into clinical practice, particularly for the prediction of response to immune-based therapies (PD-L1 expression, genetic alterations and gene-expression panels) and for monitoring relapse and disease progression (circulating DNA and CTCs). These novel markers have the potential to greatly enhance our capacity to provide effective therapy and improve patient outcomes.

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