Immune biomarkers for prognosis and prediction of responses to immune checkpoint blockade in cutaneous melanoma

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\textbf{ABSTRACT}

Existing clinical, anatomopathological and molecular biomarkers fail to reliably predict the prognosis of cutaneous melanoma. Biomarkers for determining which patients receive adjuvant therapies are needed. The emergence of new technologies and the discovery of new immune populations with different prognostic values allow the immune network in the tumor to be better understood. Importantly, new molecules identified and expressed by immune cells have been shown to reduce the antitumor immune efficacy of therapies, prompting researchers to develop antibodies targeting so-called “immune checkpoints”, which have now entered the oncotherapeutic armamentarium.

\textbf{Introduction}

Oncogenesis defines the process by which a normal cell acquires new properties allowing it to proliferate and form tumors. The essential steps in this process comprise of tumor-cell intrinsic hallmarks (e.g., cell death resistance, invasion and metastatic capacities, immortality) tumor-cell extrinsic characteristics (e.g., angiogenesis, inflammation) and, more importantly, avoiding destruction by the immune system.\textsuperscript{1} Immune cells are able to recognize and destroy transformed cells, this process termed immunosurveillance. However, immunosurveillance can result in a selective pressure, or “immunoediting”, that gives rise to resistant cancer cell clones. From immunosurveillance through to immunoediting, tumor-infiltrating immune cells play a central role in cancer progression and, accordingly, can make excellent prognostic markers.\textsuperscript{2}

The study of immune infiltrates in several pathologies,\textsuperscript{3} including ovarian cancer,\textsuperscript{4} gastrointestinal tumors\textsuperscript{5} and colon carcinoma,\textsuperscript{6} led to the establishment of a worldwide consensus called Immunoscore\textsuperscript{8} led by Jérôme Galon and Franck Pagès.\textsuperscript{7} Immunoscore\textsuperscript{8} has shown how tumor-infiltrating effector memory CD8\textsuperscript{+} T cells promote favorable clinical outcomes.\textsuperscript{8}

Due to its expression of several tumor antigens (TA), melanoma is an immunogenic cancer and is readily infiltrated by antigen-specific T cells. Since the discovery of the first melanoma antigen by Boon and colleagues,\textsuperscript{9} many groups have focused their research on TAs and have monitored the frequency of antigen-specific T cells and their prognostic relevance in this pathology. Much effort and deep analyses revealed the incapacity of these cells to mount efficient antitumor immune responses. \textit{De facto}, these antigen-specific T cells were shown to express several immune checkpoints (IC), which influenced proliferative capacity and cytokine secretion.\textsuperscript{10}

Zarour and his team observed an important expression and co-expression of ICs, including programmed cell death 1 (PD-1), B- and T-lymphocyte attenuator (BTLA), T-cell immunoglobulin mucin-3 (TIM-3) and T cell tyrosine-based inhibitory motif domain (TIGIT) on CD8\textsuperscript{+}NY-ESO-1\textsuperscript{+}-specific T cells.\textsuperscript{11-15} In the tumor bed, these T cell ICs bind their ligands, which are widely expressed by microenvironmental cells.\textsuperscript{10}

Antibodies targeting ICs have shown unprecedented efficacy against several cancers, especially metastatic melanoma.\textsuperscript{16-19} However, prognostic biomarkers of efficacy and toxicity are critically required to guide the use of these immunotherapies, prevent adverse effects, improve cost-benefit ratios, and increase the proportion of responding patients.

Preliminary results from immunohistochemistry (IHC) and flow cytometry have revealed the prognostic significance of immune cell populations. Throughout this review, we provide new insights into the function of immune cell populations in cutaneous melanoma, and how these can predict outcomes to IC blockade therapy. We also give a perspective on approaches to determine and validate novel biomarkers.

\textbf{Prognostic impact of immune cell populations in cutaneous melanoma}

Pioneering work by Clark and colleagues showed the benefit of inflammatory cells within melanomas, especially their presence at the lower skin levels of melanoma invasion.\textsuperscript{20} Subsequent studies performed qualitative analyses of melanoma infiltrates, sub-dividing infiltrates into three categories classified as “absent”, “non-brisk” and “brisk”. Both in primary
tumors and metastases, brisk infiltration of the cancer microenvironment by immune cells led to a favorable clinical outcome, particularly in the common subtype “superficial spread melanoma”. Importantly, immune cells must infiltrate the tumor bed and be present during the vertical tumor growth of the primary lesion. More recently, the clinical benefit conveyed by immune cells was shown to be an independent predictor of sentinel lymph node status, regional progression and disease-free and overall survival in patients with cutaneous melanoma.

A subsequent study reclassified immune cell infiltration of melanoma into three immunotypes, A, B and C, taking into account spatial organization within lymph node metastases. Immunotypes A, B and C were characterized respectively by no immune cell infiltration, low infiltration mainly localized around blood vessels and diffuse and broad infiltration throughout the entire tumor site. Despite its underrepresentation observed in melanomas, immunotype C correlates positively with patient survival, as previously reported. Indeed, at the time of analysis, 11 of 12 patients presenting with immunotype C were reported to be clinically free of disease after surgery.

The composition of intratumoural immune infiltrates is diverse, containing multiple populations with different functions. Hence, the presence and location of immune infiltrates alone may be too simplistic for accurate prognoses, given the complex interrelationships between immune cells.

**T lymphocytes and their prognostic value in melanoma**

T cell subsets have been among the most widely studied immune populations in melanoma pathology. T cells possess distinct, extensive functions as regulators and effectors of the tumor immune response. CD8+ T cells are often associated with a favorable clinical outcome (Table 1). These T cells have been shown to co-localize with PD-L1, a critical immune checkpoint, and major histocompatibility complex (MHC) class I molecules expressed on the surface of tumor cells in a pro-inflammatory microenvironment. CD8+ T cells exert tumoricidal functions characterized by the expression of Granzyme B, and the activating molecules CD25 and OX40. CD8+ T cells are associated with a better outcome when they are present in the peritumoural regions or in the tumor bed of primary tumors. However, one study did not confirm this positive association between the accumulation of CD8+ T cells and a favorable prognosis. One explanation for this could be the underrepresentation in their cohort of thick melanomas (>1.7 mm) compared with previous studies. In some studies, CD3+ T cells, which include CD8+ and CD4+ T cells, have also been found deleterious and associated with shorter survival. This observation could be explained by the abundance of different T lymphocyte subsets, especially within CD4+ helper T cells. Indeed, cytokines associated with the Th1 CD4+ T cell subset are strongly linked to positive clinical responses as opposed to CD4+CD25+CD127-Foxp3+ regulatory T cells (Tregs), which are usually related to a poor clinical prognosis (Table 1). The deleterious impact of Treg accumulation in melanomas has been described in several studies. Our own published findings have shown that the accumulation of Tregs in tumor beds correlates with an unfavorable prognosis in melanoma, with worse outcomes in the context of BRAF mutation. Melanomas carrying a BRAF mutation are known to have dismal prognosis, although these can be controlled by BRAF inhibitors. Interestingly, another study showed a positive association between Treg accumulation in the primary lesion and a BRAF mutation. Contrary to these findings, two studies failed to find an association between Treg infiltration of primary tumors or metastatic sites and clinical outcome, which might be due to a proportion of activated T cells known to transiently express Foxp3 in a stimulation-promoting microenvironment. Circularizing regulatory T cells have no impact on the survival of melanoma patients at any stage of the disease (ref. and Jacquelot et al., unpublished observations).

Recently, we investigated not only the prognostic impact of T cell populations, but also the specific expression of inhibitory or activating ligands/receptors, chemokine receptors and the functional status of these cells on several stage III/IV melanoma cohorts. Several parameters correlated with clinical outcome after adjusting for key clinical covariates. Notably, NKG2D, CCR9 and CXCR3 expressed on tumoral CD8+ T cells, naïve circulating CD8+ T cells and effector memory peripheral CD4+ T cells, respectively, correlated with a positive clinical outcome. In contrast with stage IV melanoma, peripheral and intratumoral CD8+ T cells in stage III patients maintain their surface expression of NKG2D. Melanoma cells are known to express NKG2D ligands, MHC class I-like ligands (e.g., MICA/B), which might explain the positive association between NKG2D and a beneficial prognosis, especially in the context of decreased tumor MHC class I expression. CCR9 expression on T cells facilitates their migration toward the gut. CCR9 also favors naïve T cell entry to the thymus. Surprisingly, in a spontaneous melanoma tumor model,

**Table 1. Melanoma prognosis depends on immune cell composition and localization.**

| Immune cells | Localization | Prognostic impact | References |
|--------------|--------------|-------------------|------------|
| Conventional CD4+ and CD8+ T lymphocytes | Primary tumor | Good and Bad | 32,35,36,87 |
| Regulatory T cells | Metastases | Good and Bad | 29,42 |
| B lymphocytes | Primary tumor | Bad/No impact | 37-39,47 |
| Macrophages | Primary tumor | Good | 29 |
| NK cells | Metastases | Bad | 29 |
| MDSC | Metastases | Good | 96 |
| Plasmacytoid DC | Metastases | Bad | 97,98 |
| Mastocytes | Metastases | Bad | 81 |
| DC | Metastases | Good | 81 |
| Neutrophils | Metastases | Bad | 81 |

In contrast with stage IV melanoma, peripheral and intratumoral CD8+ T cells in stage III patients maintain their surface expression of NKG2D. Melanoma cells are known to express NKG2D ligands, MHC class I-like ligands (e.g., MICA/B), which might explain the positive association between NKG2D and a beneficial prognosis, especially in the context of decreased tumor MHC class I expression. CCR9 expression on T cells facilitates their migration toward the gut. CCR9 also favors naïve T cell entry to the thymus. Surprisingly, in a spontaneous melanoma tumor model,
we have found the accumulation of naive CCR9\(^+\) CD8\(^+\) T cells was inversely correlated with tumor weight, thus presumably carrying a benefit. Moreover, we found a 10–100-fold greater expression of CCL25, a CCR9 ligand, in the tumor bed compared with the small intestine, potentially explaining this accumulation of CCR9\(^+\) CD8\(^+\) T cells. Antibody-mediated blockade of CCL25 favored tumor growth, highlighting its involvement in natural tumor immunosurveillance,\(^{56}\) similarly to that reported for CXCR3.\(^{56,57}\) Other groups have also found a positive association between the presence of granzyme B, CD25 and OX40 on TILs in the tumor and peritumoral areas and a favorable prognosis.\(^{32,34}\)

Besides these markers associated with positive outcomes, we also revealed parameters associated with dismal prognosis.\(^{42,50}\) On circulating T cells, we found high PD-L1 expression to be related to a worse outcome.\(^{42}\) In several solid malignancies, an accumulation of memory T cells has correlated with a positive prognosis,\(^{3,8}\) consistent with this, we found that a high prevalence of naive CD45RA\(^-\) CD4\(^+\) T cells in the metastatic lymph node is associated with a dismal prognosis. Prognosis is worse when this parameter is associated with more than three invaded lymph nodes,\(^{42}\) known to be an essential factor of disease progression.\(^{58}\)

We also found that PD-L1 exhibits an unfavourable prognostic impact in metastatic melanoma.\(^{42}\) Many studies have evaluated the expression of PD-L1 in tumors, principally on melanoma cells, with contrasting results regarding prognosis.\(^{31,39-62}\) The differences observed in these studies could be explained by differing co-localization of immune cells, which have been associated with clinical benefit by secreting type I and II interferons that upregulate PD-L1 on tumor cells as a natural protective mechanism.\(^{33,62,63}\) Other explanations could be differences in techniques for assessing PD-L1 expression, the cut-off value for the positivity, biopsy selection, or tumor lesion localization.\(^{64}\) However, our findings are in line with previous studies that suggested PD-L1 expression on circulating T cells predicts a worse survival.\(^{65}\)

Chemokine receptor expression on circulating T cells also has prognostic significance in stage IV melanoma. The expression of CCR6 and CCR10 with or without cutaneous lymphocyte antigen (CLA) on CD8\(^+\) and CD4\(^+\) T cells are correlated with a dismal prognosis.\(^{50}\) The negative impact conveyed by CCR6\(^+\)CD8\(^+\) effector memory T cells might be due to the nature of this population. This heterogeneous population is composed of Te17 T cells.\(^{66}\) In humans, Te17 cells appear to accumulate in pro-inflammatory lesions and might contribute to the pro-tumoral environment.\(^{57}\) In mice, this population contributes to antitumor immune responses that control established melanoma growth, principally resulting from enhanced IFN\(\gamma\) production \textit{in-vivo}.\(^{68,69}\) CCR10 and CLA expression on T cells imprint these cells with a skin-migratory phenotype.\(^{70}\) On melanoma cells, CCR10 expression is associated with a dismal prognosis and disease progression.\(^{71-73}\) Collectively, these results suggest that, in melanoma, high CCR10 expression either on immune or tumor cells conveys a worse clinical outcome.

In summary, the deep dissection of tumor-infiltrating and circulating immune T cell populations reveals subsets associated with favorable or unfavorable clinical outcomes in cutaneous metastatic melanoma.

### B lymphocytes and their prognostic impact on survival

B lymphocytes represent 3–5% of tumor-infiltrating cells, especially in primary tumors.\(^{74}\) However, the prognostic impact of B cells in melanoma remains unclear. Some studies have described B cells as tumor-promoting cells \textit{via} their secretion of suppressive factors,\(^{75,76}\) whereas others have described anti-tumor properties in line with their production of immunoglobulins.\(^{75,76}\) In primary tumors, B cell accumulation, both at the tumor site and in peritumoral regions, has been preferentially found to associate with prolonged patient survival.\(^{77,78}\) However, in contrast, late differentiated B cells and plasmocytes correlate with poor outcome.\(^{79}\) In metastatic lesions, unexpectedly, both B cells and plasmocytes have been associated with a prolonged overall survival\(^{29}\) (Table 1), although these results need further confirmation.

### Innate immune cells populations: Friend or foe?

Certain innate immune cell populations, notably neutrophils, macrophages and mast cells, are known to promote tumor inflammation, which in turn can sustain cancer progression.\(^{75,80}\) In stage I/II melanoma patients, neutrophil infiltration in the tumor bed and plasmacytoid dendritic cells (DC) infiltration in the stroma have been associated with dismal prognosis in cutaneous melanoma.\(^{35}\) Infiltrating DC or peritumoural DC located in the primary tumor or in metastatic lymph nodes have been shown, in most cases, to be immature, which might explain the negative impact conveyed by these cells.\(^{81,82}\) Previously, we reported a negative prognosis associated with non-T, non-NK cells in lymph node metastases, independent of clinical factors (e.g., gender, BRAF status, number of metastatic lymph nodes and disease stage). This heterogeneous population was composed of B cells, but also contained a substantial proportion of myeloid-derived suppressor cells (MDSC) from the granulocytic lineage (CD15\(^+\) CD11b\(^+\) CD33\(^+\) CD14\(^+\)).\(^{29}\) The presence of intratumoral MDSC in melanoma conveys a worse prognostic value\(^{83}\) and, moreover, the potential recirculation of intratumourally-differentiated MDSC correlates with the clinical stage of disease and metastasis.\(^{84-86}\) (Table 1).

In contrast, DC-LAMP\(^+\) DC have been correlated with a favorable clinical outcome.\(^{35}\) Ladányi and colleagues demonstrated the presence of peritumoural DC-LAMP\(^+\) cells is positively associated with overall survival in melanoma patients.\(^{87}\) These findings have been confirmed by Jensen \textit{et al.}, who have shown a positive correlation between patient survival and the presence of peritumoural DC-LAMP\(^+\) cells in stage I/II melanoma patients (Table 1). Interestingly, an association between this DC subset and infiltrating activated (CD25\(^+\) and OX40\(^+\)) T cells has been found, in which immune cells were organized as tertiary lymphoid structures conveying a favorable clinical outcome.\(^{67}\)

Macrophages can be characterized into two major populations, termed M1 and M2, as a result of the tumor microenvironment, inducing polarization. M1 macrophages are characteristically antitumorigenic, whereas M2 macrophages are typically pro-tumorigenic. These macrophage subpopulations may be defined by their co-expression of CD68 with...
NOS2 or CD163, the latter marker appearing more specific to the M2 population. In melanoma, intratumoural macrophages are typically associated with an unfavourable prognosis.42 The evaluation of more than 200 cases of stage I/II cutaneous melanoma has revealed that the presence of CD68+ cells localized to the tumoral invasive front forms a negative prognostic value for overall survival irrespective of thickness and ulceration status88 (Table 1). In addition, a univariate analysis revealed that this CD68+ population, when localized to the tumor bed, had a negative impact on overall survival.88 A recent study has confirmed this negative impact conveyed by intratumoural macrophages, as well as an association with metastases formation89 (Table 1).

NK cells form an immune cell population with specific functions that lie in between innate and adaptive immunity. NK cells recognize cancer cells through a variety of surface-expressed activating and inhibitory receptors, but may also gain memory-like functionality, usually driven by B and T lymphocytes.90 These key functions constitute their critical biologic roles in immunosurveillance. We have demonstrated the crucial role of NK cells in melanoma.91 Melanoma cells can down-regulate expression of MHC class I and increase expression of certain activating ligands, such as MICA/B.52 Modulations in the balance of cancer cell surface-expressed molecules facilitate NK cell activation, for example, through engagement of NKG2D, an NK cell activating receptor, with upregulated MICA/B. Nonetheless, in cutaneous melanoma, NK cells can have altered surface molecule expression levels, notably down-regulated Nkp46 and NKG2D (activating receptors), leading to decreased cytolytic activity.92,93 Nkp46 expression on NK cells is reduced in patients with metastatic disease, and higher Nkp46 expression levels are positively correlated with clinical outcome.91 Interestingly, chemotherapy can counteract this phenomenon and promote Nkp46 expression on NK cells.94,95 In primary tumors, NK cells are mainly found in the peritumoural margin and not in the tumor mass.96 Recently, a unique NK cell population has been identified in metastatic lymph nodes, characterized by high CD56 and CD16 co-expression. These NK cells appear to lyse tumor cells more efficiently than blood-derived NK cells in the presence of IL-2 and IL-15.97,98 Beside these findings, Ali and colleagues have described the enrichment of a CD57+CD56dim NK cell population in tumor-infiltrated lymph nodes, which positively correlates with patient outcome.98

Despite promising results, most of the parameters described above need to be validated as prognostic indicators for new targeted therapies and immunotherapies. The validation or discovery of biomarkers that predict treatment activity and toxicity will markedly improve the response rates to these regimens, especially for immune checkpoint blockers (ICBs).

**Pharmacodynamic and predictive markers of IC blockers**

ICs regulate the activity and functions of immune cells. First described during chronic infections, the regulatory role of ICs was later assessed in the context of tumor immunosurveillance. For instance, PD-1, a well-characterized IC, is expressed at high levels by intratumoural T cells and has the principal function to inhibit TCR signaling.42,99,100 The expression of such ICs at the surface of cells allowed researchers to develop monoclonal antibodies targeting these molecules, termed as IC blockers.

Ipilimumab and tremelimumab are ICs targeting cytotoxic T-lymphocyte associated antigen-4 (CTLA-4), and were the first ICs to demonstrate clinical benefit in metastatic melanoma.101,102 The superior efficacy of ipilimumab treatment compared with TA vaccination16 and when combined with dacarbazine17 definitively proved the efficacy of CTLA-4 blockers, and led to FDA approval of ipilimumab for treatment of unresectable or metastatic melanoma in 2011. This breakthrough in melanoma treatment was followed by the approval of the PD-1 blockers pembrolizumab and nivolumab in 2014 for unresectable metastatic melanoma resistant to standard treatment103. Recently, the approval of ipilimumab therapy was extended to stage III melanoma patients after complete resection following evidence of increased recurrence-free and overall survival.104,105

Unfortunately, the exceptional efficacy of ICs is accompanied by immune-related adverse effects.106 Indeed, although several clinical trials combining PD-1 and CTLA-4 blockers have confirmed superior efficacy compared with anti-PD-1 and anti-CTLA-4 monotherapies,107,108 the greater toxicity of this combination has, for the time being, precluded continuation of this strategy.109 The fact that 50% of patients do not respond to this regimen has driven the search for surrogate and predictive markers of clinical response and toxicity. Furthermore, despite overcoming the primary resistance, the majority of patients who benefit from PD-1, CTLA-4 or their co-blockade will relapse. Hence, biomarkers for secondary resistance are also needed to help guide therapy decisions.109 A number of past publications have highlighted and made progress in solving this challenge, these extensively detailed elsewhere.64,110-113 We will focus on the most recent findings.

Patients benefiting from ipilimumab have increased absolute numbers of peripheral lymphocytes and have delayed increases in CD4+ and CD8+ T cell populations during treatment, both, which were found to be correlated with a positive outcome.114 Low lactate dehydrogenase (LDH; an enzyme reflecting tumor burden), MDSC and absolute lymphocyte counts, and high absolute eosinophil, Treg, and relative lymphocyte counts are all associated with a favorable overall survival following ipilimumab.115 Peripheral γδ T cells have also been shown to be associated with longer survival during ipilimumab treatment. High proportions of circulating Vα1+ Vδ1 T cells appear to identify shorter overall survival, whereas Vα2+ Vδ2 T cells correlate with longer survival.116 We previously demonstrated the negative impact of high basal serum levels of soluble CD25 and LDH on metastases-free survival and overall survival.117,118 In 2015, Koguchi et al, showed that high basal serum levels of CXCL11 and sMICA are associated with shorter overall survival in ipilimumab-treated patients. They also confirmed soluble CD25 and LDH are associated with shorter overall survival in their cohort.119 Recent evidence has shown CLA to be preferentially expressed on CD8+ effector memory T cells from patients responding to CTLA-4 blockade.30 Furthermore, a recent study suggests sensitivity to CTLA-4 blockade is accompanied by a significantly higher intratumoural density of CD8+ T cells during the early phase of treatment.120
Several studies also revealed various immune biomarkers associated with anti-PD1 efficacy. High baseline CD3 levels by IHC and CD45RO+CD8+ T cells located in the tumor mass were associated with reduced tumor burden during anti-PD1 therapy after ipilimumab treatment failure.120 In contrast, a previous study has claimed a predictive impact of these parameters when localized in the invasive margin, but not in the tumor center.99 Moreover, proliferating, granzyme B+ CD8+ T cells were localized in the tumor bed together with PD1-, PD-L1- and LAG-3-expressing cells on treatment, demonstrating a pro-inflammatory environment associated with a therapeutic response.33,63,99,120,121 Not only the relative T lymphocyte count, but also the relative eosinophil count, metastases locations and LDH ratio (value divided by the upper limit of normal) were biomarkers associated with objective responses and overall survival under pembrolizumab therapy. In stage III/IV patients, low metastatic burden, a LDH-ratio ≤ 2.5 and high relative lymphocyte and eosinophil counts were shown to be strongly associated with favorable prognosis.122

Taken together, the definition of a combinatorial model composed of these predictive parameters led to the development of a nomogram for assessing the predictive efficacy and clinical impact of pembrolizumab and ipilimumab in metastatic melanoma patients.115,122

During treatment, resistance to ICBs can appear. This can be mediated in part by upregulation of other ICs at the surface of T lymphocytes, or via loss of PTEN expression by tumor cells, which allows for PI3K/Akt pathway activation.123,124 For instance, TIM3 can be overexpressed at the surface of CD8+ T cells, where it can bind its corresponding ligands expressed in the tumor microenvironment, resulting in downregulated tumoricidal effector functions.123 PTEN loss, which causes PI3K/Akt activation, can also lead to the activation of transcription factors that regulate epithelial–mesenchymal transition, cellular adhesion, angiogenesis and PD-L1 expression at the surface of tumor cells.124 Other mutations targeting JAK1/2 or the β2m genes in melanoma cells reduce sensitivity to the anti-proliferative effect of interferons and can result in defects to the antigen-presenting machinery leading to the resistance of the anti-PD1 therapy.125,126 Moreover, prolonged exposure to interferons favors the overexpression of T cell inhibitory receptors, including PD1, LAG3 and TIGIT, which can blunt immune responses reactivated by ICB therapies.127

The identification of the aforementioned biomarkers represents a significant breakthrough in immunotherapy. Nevertheless, a global approach gathering -omics methods together with IHC and flow cytometry should help the scientific community to discover and validate suitable parameters for melanoma treatment prognosis.

**Perspectives: Systems biology approaches**

Identifying more accurate prognostic indicators of ICB responses requires a better understanding of the interplay between tumors and tumor-extrinsic parameters. Due to their dramatically decreased cost and improved accessibility, computational systems biology approaches are increasingly used to understand almost all aspects of medical research. However, despite improved robustness of data analysis and annotation, systems biology technologies can still sometimes yield erroneous findings. Joint modeling and integration of data from heterogeneous sources may be able to address these points. Such measures can help self-correct erroneous conclusions, confirm relationships and highlight clinically relevant changes that might have been missed when looking at methods individually. Accepted semantic resources and ontologies are required for the efficient and coordinated handling of data of various origin and structure.

Etiology, diagnosis and cure of metastatic melanoma could benefit from the so-called “big data” revolution in medical research and healthcare. Digitalization of patient registries and the mass use of personal portable devices could be used to efficiently monitor adverse events from ICBs and patient comfort during their therapy. Harnessing these factors may also provide quantitative, longitudinal information on a wide range of lifestyle, physiologic and environmental parameters. These could complement existing phenotypic data derived from molecular biology or genetics, which are routinely monitored in metastatic melanoma patients.

Peripheral blood LDH levels and BRAF mutation status are examples of melanoma parameters that could benefit from systems biology approaches that combine data from IHC or flow cytometry with epigenomics, transcriptomics, proteomics and metabolomics. Initial global analyses in melanoma were focused on large data sets with exome or gene expression profiling, which revealed recurrent mutations in melanoma128,129 and genes associated with clinical outcome.130 Other studies focused on small cohorts with combinatorial approaches and demonstrated the importance of immune-specific gene signatures to clinical outcome,131 or an integrative genomic approach for the identification of genes associated with the ulceration status.132

In 2013, a pan-cancer analysis initiated by the Cancer Genome Atlas Network made significant advances through studying 12 tumor types using systems biology approaches.133 In 2015, the network published an article using DNA, RNA and protein-based analyses on 333 cutaneous melanomas, mainly collected from regional and distant metastases and matched with peripheral blood samples.134 They classified the disease into four subtypes according to mutational status (BRAF, NRAS, NF1 and triple wild-type) and uncovered mutational immune-associated profiles that could help clinical and therapeutic decision-making.134 Of note, the study confirmed the important role of the immune system in gene signatures associated with improved overall survival, irrespective of the mutational status.134 In particular, LCK-expressing, enriched tumors and a high lymphocyte score (determined by a semi-quantitative count of lymphocytes in a sample) were associated with favorable patient outcomes.134 Nonetheless, several aspects were not investigated, notably the determination of the neoantigen landscape in these tumors. It is well-known that melanoma possesses the strongest neoantigen repertoire across human cancers135 which results in an elevated fraction of specific antitumor T cells expressing PD-1 and CD137.136

The gut microbiota may also play a significant role in the response to ICBs.137,138 Certain gut microbiota in mice were found to be essential for anti-PD1 responsiveness138 and, moreover, fecal microbial transplantation of feces from patients
responding to ipilimumab restored the efficacy of anti-CTLA-4 blockade in germ-free tumor-bearing mice.137

Collectively, these results highlight the importance of studying tumor biology via global approaches. These combinatorial approaches should pave the way to personalized medicine, taking into account an individual’s genetics and environmental influences.

Conclusions

The prognostic metastasis cutaneous melanoma has been greatly improved through the emergence of targeted therapies and immunotherapies. Despite consensus to the benefit of studying immune infiltrates in melanoma, no prognostic markers were included by Balch CM et al. for the American Joint Cancer Committee in the last published version18 of melanoma criteria and prognostic factors. Nevertheless, a cooperative coordination on immune monitoring should yield more accurate prognostic indicators that can be used to guide treatment decision-making in cutaneous metastatic melanoma.

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