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Emerging Tissue and Blood-Based Biomarkers that may Predict Response to Immune Checkpoint Inhibition

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Abstract

The immune system plays an essential role in the surveillance and eradication of neoplastic cells. This interaction is modulated via immunologic regulators (checkpoints). Antibodies that block the checkpoints cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and the programmed cell death protein 1 pathway (PD1/PD-L1) have demonstrated efficacy in a number of malignancies. However, response rates are variable, and administration of these antibodies can be associated with immune-related adverse events. Therefore, researchers are engaged in an effort to discover biomarkers that may predict response to these agents. This review focuses on potential blood and tumor-based biomarkers that have been assessed in patients treated with these checkpoint-blocking antibodies.

Keywords

Immunotherapy; Checkpoint blockade; Ipilimumab; Nivolumab; Pembrolizumab; Biomarkers; PD-1; PD-L1; CTLA-4; Neoantigens; Melanoma

Introduction

The immune system plays an essential role in the surveillance and eradication of neoplastic cells. This interaction is modulated via immunologic regulators (checkpoints). Over the past 4 years, antibodies that block the checkpoints, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and the programmed cell death protein 1 pathway (PD1/PD-L1) have demonstrated efficacy in a number of malignancies, including metastatic melanoma (MM) [1–4], non-small cell lung cancer (NSCLC) [5, 6], and renal cell carcinoma, among others [7, 8]. However, response rates can vary widely, and administration of these classes of drugs may be associated with a number of immune-related adverse events. Hence, there is an

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ongoing effort to identify biomarkers which may identify patients most likely to benefit from checkpoint blockade to spare others treatment-related toxicity. This review focuses on potential blood and tumor-based biomarkers that have been assessed in patients treated with these checkpoint-blocking antibodies. Due to the many potential biomarkers that have been investigated, we have organized our review into three sections. We first discuss potential biomarkers identifiable in the peripheral blood through common clinical laboratory tests. We then focus upon potential immunologic biomarkers in the peripheral blood. Finally, we discuss biomarkers related directly to the tumor or tumor microenvironment.

Potential Biomarkers in the Peripheral Blood Identified Through Routine Clinical Testing

Patients with cancer undergo extensive monitoring of their peripheral blood as part of routine clinical care. Many biomarker investigations have therefore focused upon examining these clinical laboratory parameters to see if any correlate with outcomes in patients receiving checkpoint inhibition. The neutrophil to lymphocyte ratio (NLR) has been found to be prognostic of survival in many solid tumor types. In a recent meta-analysis, a NLR > 4 was associated with an overall survival (OS) hazard ratio (HR) of 1.81 (95 % CI= 1.67 to 1.97, $P < .001$); this was observed in all disease subgroups, sites, and stages [9]. In patients with MM treated with ipilimumab, the baseline NLR was also found to be associated with OS; patients with a NLR ≥ 4 at baseline had a poorer OS compared to those with an NLR < 4 at baseline (HR = 2.79; 95 % CI 1.49–5.23, $P = 0.001$) [10]. This was confirmed in an Italian study of patients with MM treated with ipilimumab, in whom a baseline NLR ≤ 5 had a significantly improved progression free survival (PFS) (HR = 0.38, 95 % CI 0.22–0.66, $P = 0.0006$) and OS (HR = 0.24, 95 % CI 0.13–0.46, $P = 0.0001$) compared to patients with NLR > 5 [11]. Whether the associations between NLR and clinical outcomes are related to ipilimumab or instead reflective of a general prognostic correlation between NLR and OS, remains unknown.

Similarly, the association between absolute lymphocyte count (ALC) and OS in patients with MM treated with ipilimumab has been noted [12, 13]. Lymphocytes have been a cell population of interest since ipilimumab targets T cells, and ALC is an easily obtainable value from the peripheral blood. In a study of 73 patients with MM, an ALC > 1000 cells/μL at the time of the second ipilimumab infusion was associated with a significant increase in OS (11.1 vs 4.8 months, log-rank test $P < .0001$). OS was also improved when the ALC increased by >200 between the first and second infusion ($P = 0.037$).

Similar results have been seen in many other studies. In a large retrospective review of six studies of ipilimumab, mean ALC increased significantly over time ($P < .001$ to $P = .03$), consistent with a pharmacodynamic effect of CTLA-4 blockade. Patients who had a greater change in ALC from baseline to week 7 or and ALC ≥ 1000 after two dose of ipilimumab had an improvement in OS ($P = .003$), However, an overall survival benefit from ipilimumab compared to the gp100 peptide vaccine control was seen regardless of rate of change of ALC [14]. ALC likely reflects a prognostic biomarker, rather than one that can specifically be used to select patients for ipilimumab treatment.

ALC has not been found to significantly correlate to response to either PD-1 inhibitor monotherapy [15] or combination therapy with nivolumab and ipilimumab [16]. The studies that correlated ALC with outcomes following PD-1 monotherapy or nivolumab + ipilimumab combination therapy correlated ALC with response outcomes as opposed to overall survival. It is possible that correlations between ALC and overall survival will be seen in patients treated with PD-1 agents as longer-term overall survival data matures.

There also appears to be a correlation between absolute eosinophil count (AEC) and response to checkpoint blockade. An increase in AEC >100 between baseline and second ipilimumab infusion in patients with MM was associated with an improved median OS (11.3 vs 6.8 months, $P = 0.012$). Similarly, in patients with MM treated with PD-blocking antibodies, an increase in AEC of 100/mm^3 at week 3 over baseline or an elevated AEC (>400/mm^3) at week 12 has been found to be associated with superior response rates, longer PFS, and OS [17].

Lactate dehydrogenase (LDH) is a known poor prognostic factor in patients with melanoma and is negatively associated with outcomes following ipilimumab. Simeone and colleagues noted that in patients with MM who were treated with ipilimumab, the proportion of patients with an LDH greater than 1.1× the upper limit of normal (ULN) decreased between baseline and week 12 among patients with disease control and increased in patients with progressive disease ($P < 0.0001$) [12]. A baseline elevated LDH was also significantly associated with poor OS [18]. There is now an externally validated prognostic nomogram model based on baseline LDH and absolute neutrophil count which calculates 6, 12, and 24-month survival probabilities in patients with MM treated with ipilimumab [19]. Similar to the experience with the NLR and ALC, which may just be reflective of general prognostic characteristics, additional research is necessary before being able to apply the prognostic nomogram to selection of patient treatment, since none of these routine peripheral blood laboratory parameters has been established as a predictive biomarker for ipilimumab treatment.

Immunologic Biomarkers in the Peripheral Blood

Several studies have investigated antigen specific T cell or serologic responses to determine if the functionality of these immune system components is relevant to outcomes with ipilimumab. NY-ESO-1, a cancer-testis antigen, has limited expression on normal tissue but is frequently expressed on malignant cells, including one-third to one-fourth of all melanoma, lung, esophageal, liver, gastric, prostate, ovarian or bladder cancers.[20] As such, it is a logical target for immunologic response and the presence of circulating antibodies or T cells targeted against NY-ESO-1 has been associated with a superior clinical response to ipilimumab in some but not all studies. In a retrospective series of 15 patients with MM, five had NY-ESO-1 antibodies and all derived clinical benefit from ipilimumab while none of the patients who had progressive disease were antibody-positive. All five antibody-positive patients also had CD4+ and CD8+ T cells directed against NY-ESO-1 after treatment [21]. This was explored in larger cohort of 144 patients with MM who received ipilimumab. Of these, 22 (16 %) were seropositive at baseline and 31 (22 %) after treatment. Patients who were seropositive had a greater likelihood of experiencing clinical benefit from ipilimumab compared to seronegative patients ($P = 0.02$, relative risk = 1.8, two-tailed Fisher test).

Patients who were seropositive and had associated CD8+ T cells were more likely to derive clinical benefit compared to those without a T cell response; they also had a significant survival advantage [22]. Another study, however, showed no obvious correlation between the presence of NY-ESO-1 antibody and outcomes following CTLA-4 blockade [23], so additional research is warranted. Further complicating these analyses is that the correlation between NY-ESO-1 and long-term outcome may be independent of ipilimumab treatment. In patients with MM, the presence of T cells responding to NY-ESO-1 was significantly associated with survival regardless of treatment [24].

Inducible co-stimulator (ICOS) is a T cell-specific molecule that belongs to the CD28/CTLA-4 family and is expressed after T cell activation [25]. ICOS was first identified as a potential biomarker for ipilimumab in a study of neoadjuvant ipilimumab administered to patients with localized bladder cancer. Expression of ICOS on circulating CD4 T cells and on T cells in tumor tissues increased after ipilimumab in all six patients treated ($P < 0.05$) [26]. In another study of MM patients who received ipilimumab, the majority of patients had an increase in the frequency of CD4 + $ICOS^{hi}$ T cells at week 7 ($P = 0.0012$) and 12 ($P = 0.0006$) after starting treatment. Sustained increase of these T cells at week 12 was associated with significantly higher rates of clinical benefit compared to those without a sustained increase ($P = 0.004$) [27]. The question of whether ICOS+ T cells could be used as a predictive biomarker for response for ipilimumab was then assessed in MM patients. After two doses of ipilimumab, the cytometry assay was found to have an estimated specificity, defined as the test's ability to correctly detect patients without a response, of 96 % (95 % CI 88–100) and sensitivity, defined as the test's ability to correctly detect patients with a response, of 71 % (95 % CI, 54–85) [28]. There is little data for changes in ICOS expression associated with administration of PD-1 blocking antibodies, although increases in the number of CD4 and CD8 T cells expressing ICOS in patients treated with a combination of nivolumab and ipilimumab have been seen [29].

Given that T cells are likely central to mechanistic responses to ipilimumab, there has been interest in the role of T cell receptor (TCR) diversity and clonality with respect to outcomes following immune checkpoint inhibition. In a study of 21 patients receiving the CTLA-4 blocking antibody tremelimumab, Robert and colleagues used next-generation sequencing to study the complementarity-determining region 3 (CDR3) from the rearranged TCR variable beta in peripheral blood mononuclear cells (PBMCs). After receiving tremelimumab, there was a median of 30 % increase in unique sequences in 19 out of 21 patients, but this did not differentiate between responders and non-responders [30]. This was explored further in cohorts of patients with castrate-resistant prostate cancer, MM and healthy controls, again using next-generation sequencing of the TCR-β genes from PBMCs. Both the prostate and MM cohorts demonstrated increased turnover in TCR clonotype frequencies and diversity when compared to baseline and to healthy controls ($P = 0.005$). While there was no significant association between changes in T cell repertoire and clinical response, improved OS was associated with maintenance of high frequency-clones present at baseline. This suggests that the presence of pre-existing high-avidity T cells may be important for anti-tumor response [31]. In a small group of patients treated with ipilimumab, one additional study investigated whether pre-treatment TCR diversity, as assessed by a PCR assay, was associated with clinical outcomes in MM patients. In this study, patients who derived clinical

benefit from ipilimumab had a higher degree of baseline T cell diversity; however, there was no correlation between TCR diversity and OS [32].

Myeloid-derived suppressor cells (MDSC) are an immunosuppressive cell population that negatively regulates adaptive and innate immunity [33, 34]. While there is some controversies over phenotypic descriptions of the MDSC population, they are commonly characterized as CD14+ CD11b+ HLA-DR$^{-/low}$ cells [35]. In a cohort of 94 patients with MM, patients with higher numbers of circulating MDSC had a shorter median OS, 8 months vs 13 months ($P < 0.001$). There was a significant inverse relationship between NY-ESO-1-specific T cells and number of circulating MDSC ($P = 0.015$) and a similar, albeit not significant, relationship between Melan-A-specific T cells and MDSC ($P = 0.092$) [24]. In a neoadjuvant study of ipilimumab, a greater decrease in circulating MDSC was associated with improved PFS ($P = 0.03$), and there was a significant decrease in MDSC after treatment with ipilimumab [36]. In a different cohort of patients treated with ipilimumab, there were no changes in MDSC frequencies over the course of treatment but patients with lower pre-treatment levels of circulating MDSC were more likely to drive clinical benefit from ipilimumab ($P < .05$) [37]. In patients treated with the combination of ipilimumab and nivolumab, objective responses with a greater than 80 % reduction in tumor burden at 12 weeks were seen in patients with a low frequency of pretreatment MDSC; no objective responses were seen in patients with high-circulating MDSC [38].

Tumor Microenvironment

Since the immunogenicity of the tumor is thought to be related to outcomes of immune checkpoint inhibition, many studies have examined the tumor or tumor microenvironment to see if particular characteristics correlate with clinical outcomes to checkpoint inhibitors. Perhaps the biomarker that has received the greatest attention is expression of the ligand for PD-1, PD-L1. While PD-L1 expression has been associated with more favorable response rates to PD-1/PD-L1 agents, PD-L1 is not a static biomarker capable of binary discrimination of responsiveness. In the first phase I trial with nivolumab, administered in patients with a variety of solid tumor types, PD-L1 was first demonstrated to associate with responses to nivolumab [39]. Grosso and colleagues expanded on these findings by performing a retrospective review of patients with non-small cell lung cancer (NSCLC) and MM who had received nivolumab. In the MM cohort, patients appeared to derive clinical benefit from nivolumab regardless of tumor PD-L1 expression, although patients with tumors higher in PD-L1 expression had more substantive clinical activity with a median OS of 21.1 vs 12.5 months [40]. Whether PD-L1 expression on the tumor cells themselves or immune cells in the tumor microenvironment is most relevant also remains unclear. In patients treated with atezolizumab (MPDL3280A), a PD-L1 blocking antibody, PD-L1 expression on tumor infiltrating lymphocytes (TILs) was significantly associated with response to therapy in patients with NSCLC. This appeared to be a more important response correlate than tumor cell PD-L1 expression, but the relative impact of PD-L1 expression on tumor cells vs. tumor infiltrating immune cells may vary across tumor types [41••]. PD-L1 expression has been examined in a number of tumor types, including MM, bladder cancer, NSCLC, and gastric cancer, and while there is consistently an association between PD-L1

expression and improved response rates, PD-L1 does not appear to consistently predict likelihood of benefit to single-agent PD-1/PD-L1 blockade (see Table 1).

The significance of PD-L1 as a possible biomarker is also unclear in patients receiving the combination of ipilimumab and nivolumab. In most studies of this combination regimen, response rates to the combination have been similar, regardless of PD-L1 status [42–44]. In a subset analysis of the phase 3 Checkmate 67 trial, which compared ipilimumab vs nivolumab vs the combination of both agents, a difference in PFS between combination immunotherapy and nivolumab monotherapy was most apparent for patients with PD-L1-negative tumors [44], Nonetheless, patients with PD-L1-positive tumors also had a higher response rate with the combination compared to nivolumab monotherapy. We therefore do not yet believe PD-L1 status can be used for clinical decision-making to select patients for combination vs. single-agent immune checkpoint inhibition. Some patients may have some tumors that are PD-L1-positive and others that are PD-L1-negative, further complicating research on this potential biomarker [45].

The predictive role of tumor infiltrating lymphocytes (TILs) as they pertain to outcomes following checkpoint inhibitors was first examined by Hamid and colleagues, who obtained pre- and on-treatment biopsies from MM patients receiving ipilimumab. In comparing patients who derived clinical benefit from those who did not, 57.1 % of those who benefited had a post-treatment increase in TILs and none had a decrease. In contrast, only 10 % of those who did not benefit had an increase in TILs and 15 % had a decrease. ($P = 0.005$). In patients with MM treated with pembrolizumab, patients who derived clinical benefit from treatment had higher CD8+ T cell densities at the invasive tumor edge compared to those who did not derive benefit. Moreover, serially sampled tumors exhibited an increase in CD8 + –cell density in the response group but not in the progression group. (Spearman's correlation $r = 0.71$, $P < 0.001$). Overall, the response group was associated with significantly higher numbers of CD8+, PD-1+ and PD-L1+ cells at both the invasive margin and the tumor center when compared to the progression group [46••].

The role of gene expression profiling in predicting response to immunotherapy has also been explored in patients treated with both CTLA-4 and PD-1 blocking antibodies, hypothesizing that patients with upregulation of genes related to the immune response are more likely to derive benefit from immunotherapy. This was explored in a prospective phase II trial of patients with MM treated with ipilimumab. Gene expression profiling (GEP) was performed on tumor biopsies obtained both pre-treatment and 3 weeks after the first infusion. As expected, patients who derived clinical benefit from ipilimumab had upregulation of 170 genes on their pretreatment tumor biopsy, most of which were involved in the immune response. In the on-treatment tumor biopsies, when compared to the prior biopsy, there were significant increases in expression of genes related to ICOS signaling in T helper cells and T helper cell differentiation as well as CTLA4 signaling [47]. In another prospective phase II trial of patients with MM treated with ipilimumab, mRNA expression profiles of tumor biopsies obtained both pre- and post-treatment were examined. Again, there were significant increases in post-treatment expression for various immune-response genes, including granzyme B and perforin 1; genes with decreased expression included the known melanoma antigen tyrosinase-related protein-2 (DCT). There was no significant difference in gene

expression profiles between patients who received 3 mg/kg or 10 mg/kg of ipilimumab, suggesting an absence of dose-response [48]. In patients with MM who were treated with pembrolizumab, GEP was performed using the NanoString nCounter platform using RNA extracted from pre-treatment biopsies. Two pre-specified signatures, the interferon-gamma 10-gene and the expanded-immune 28-gene, were utilized. Both signatures demonstrated statistically significant associations with both ORR ($P = 0.047$ and 0.027, respectively) and PFS ($P = 0.016$ and 0.015) [49].

Melanoma and non-small cell lung cancer have been shown to have the highest somatic mutation prevalence across human cancer subtypes, likely secondary to chronic mutagen exposure (i.e., ultraviolet radiation, cigarette smoke) [50], Given these data, it was then hypothesized that patient-mutated epitopes, known as neoantigens, may play an important role in the T cell response driven by checkpoint blockade. This was first assessed by van Rooij and colleagues who published a case report of a patient with stage IV melanoma who derived clinical benefit from ipilimumab treatment. They performed tumor whole-exome sequencing which revealed 1657 somatic mutations. Using a bioinformatics platform, the investigators derived 448 potential CD8 T cell epitopes that were analyzed for reactivity against the patient's TILs. This demonstrated a dominant response against a mutated epitope in the ATR kinase. Mutated ATR-specific T cells were present before initiating ipilimumab therapy and expanded fivefold during therapy [51].

This hypothetical relationship between mutation load and clinical benefit from immunotherapy was explored further in two additional cohorts, one consisting of patients with MM and the other with metastatic NSCLC. In patients with MM, mutational load was associated with the degree of clinical benefit from ipilimumab but was not sufficient to predict benefit. Again, predicted neoantigen activated T cells in situ from the patients treated with ipilimumab [52••]. In the lung discovery cohort ($n = 16$), a higher somatic nonsynonymous mutation burden was associated with clinical efficacy of pembrolizumab (Mann-Whitney $P = 0.02$). In the entire set of sequenced tumors ($n = 34$), the PFS was higher in patients with a high nonsynonymous burden vs a low nonsynonymous burden (median PFS 14.5 vs 3.7 months, log-rank $P = 0.01$, HR 0.19, 95 % CI 0.05–0.70). Using a cut point of ≥178 nonsynonymous mutations, patients in the discovery cohort with a higher burden had a likelihood ratio for deriving clinical benefit of 3.0; the sensitivity was 100 % (95 % CI 59–100), and the specificity was 67 % (29–93). In the validation cohort, the sensitivity and specificity were 86 and 75 %, respectively [53]. These findings raise interesting hypotheses, but additional research is necessary to confirm these early associations.

Conclusions

As discussed above, there are a number of both peripheral blood and tumor-based laboratory tests that have been examined as potential biomarkers to predict response to either CTLA-4 or PD-1/PD-L1 blockade. Unfortunately, although a number of assays have been associated with outcomes, none have been prospectively validated to predict likelihood of treatment benefit to enable patient selection. Given the complexities of the immune system, it is quite likely that immunologic biomarkers will have different meanings than traditional biomarkers

such as specific oncogenes that can be targeted with small molecule inhibitors. Immunologic biomarkers provide mechanistic insights into the ways immune checkpoint antibodies may work and help identify populations of patients with better and worse outcomes which may ultimately justify combination therapy for certain patient populations. As the field moves forward, it is essential that researchers incorporate new assays into clinical trial design in order to improve the treatment of patients with these agents.

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Table 1

Response to immunotherapeutic agents stratified by tumor PD-L1 staining

	ST [39]	MM [54]	MM [40]	NSCLC [55]	NSCLC [56]	MM [57]	H&N [58]	MM [59]	ST [41••]	MM [60]	NSCLC [61]	Bladder [62]	ST [63]	NSCLC [64]	H&N [65]
	Nivolumab				Pembrolizumab				Atezolizumab				MEDI4736		
n=	**42**	**44**	**34**	**63**	**146**	**113**	**55**	**411**	**103**	**30**	**53**	**67**	**179**	**200**	**62**
Overall response rate	21 %	32 %	29 %	14 %	19 %	40 %	18 %	40 %	21 %	23 %	23 %	26 %	11 %	16 %	11 %
PD-L1 −	0%	19 %	17 %	16 %	11 %	13 %	11 %	13 %	13 %	20 %	15 %	11 %	4%	5%	8%
#PD-L1 +	36 %	67 %	44 %	13 %	37 %	49 %	46 %	49 %	36 %	27 %	46 %	43 %	22 %	27 %	18 %

#PD-L1 positive was defined using different cutoffs and assessed by different antibodies within many of the different clinical trials. Please refer to the primary citations for specifics

ST solid tumors, *MM* metastatic melanoma, *NSCLC* non-small cell lung cancer, *H and N* head and neck cancer