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REVIEW

Circulating biomarkers predictive of tumor response to cancer immunotherapy

Ernest Y. Lee[a,b,c] and Rajan P. Kulkarni[d,e,f]

[a]Department of Bioengineering, UCLA, Los Angeles, CA, USA; [b]Department of Dermatology, UCLA, Los Angeles, CA, USA; [c]UCLA-Caltech Medical Scientist Training Program, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; [d]Department of Dermatology, OHSU, Portland, OR, USA; [e]Cancer Early Detection and Advanced Research Center (CEDAR), Knight Cancer Institute (KCI), OHSU, Portland, OR, USA; [f]Division of Operative Care, Portland VA Medical Center (PVAMC), Portland, OR, USA

ABSTRACT

Introduction: The advent of checkpoint blockade immunotherapy has revolutionized cancer treatment, but clinical response to immunotherapies is highly heterogeneous among individual patients and between cancer types. This represents a challenge to oncologists when choosing specific immunotherapies for personalized medicine. Thus, biomarkers that can predict tumor responsiveness to immunotherapies before and during treatment are invaluable.

Areas covered: We review the latest advances in 'liquid biopsy' biomarkers for noninvasive prediction and in-treatment monitoring of tumor response to immunotherapy, focusing primarily on melanoma and non-small cell lung cancer. We concentrate on high-quality studies published within the last five years on checkpoint blockade immunotherapies, and highlight significant breakthroughs, identify key areas for improvement, and provide recommendations for how these diagnostic tools can be translated into clinical practice.

Expert opinion: The first biomarkers proposed to predict tumor response to immunotherapy were based on PD1/PDL1 expression, but their predictive value is limited to specific cancers or patient populations. Recent advances in single-cell molecular profiling of circulating tumor cells and host cells using next-generation sequencing has dramatically expanded the pool of potentially useful predictive biomarkers. As immunotherapy moves toward personalized medicine, a composite panel of both genomic and proteomic biomarkers will have enormous utility in therapeutic decision-making.

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Checkpoint blockade immunotherapy; liquid biopsy; noninvasive diagnostics; circulating tumor cells; personalized medicine

1. Introduction and organization of review

The explosion of research into checkpoint blockade immunotherapies (CBI) is owed to their resounding clinical success in dramatically increasing survival rates across multiple cancer types, most notably metastatic melanoma which historically has had an extremely poor prognosis. In recognition of this achievement, James P. Allison and Tasuku Honjo jointly received the 2018 Nobel Prize in Physiology or Medicine for their fundamental contributions to the discovery of CBI. The list of cancers with FDA-approved indications for CBI now include metastatic melanoma, non-small cell lung cancers, renal cell carcinoma, head and neck squamous cell carcinoma, and bladder cancer, with a multitude of other tumor-therapy combinations under investigation in ongoing clinical trials.

Despite the success of CBI, several barriers still exist in extending clinical benefit to a greater number of patients. Although checkpoint immunotherapies work well for patients that do achieve clinical responses, a subset of patients do not respond or respond poorly to the same treatment. At present, it is not well understood how and why this occurs. Recent work has implicated dynamic changes in both host immunology as well as heterogeneities in tumor genetics and microenvironment. In addition, there is no consensus as to which objective metrics best enable prediction of clinical response. Identification of such metrics would enable oncologists to choose specific therapies before initiation, and potentially adapt and modify therapeutic strategies as they are monitored throughout therapy. Biomarkers reflecting tumor immune microenvironment and tumor cell-intrinsic features, obtained directly from tumor samples, have been studied as potential markers of response to CBI. Examples include intratumor PDL1 expression, density of tumor-infiltrating lymphocyte (TIL), tumor mutational burden (TMB) [1], tumor transcriptomics [2,3], and tumor mismatch-repair (MMR) deficiency [4], which have been shown to predict treatment effects of CBI [5]. However, these biomarkers require invasive sampling and are not practical from a risk-benefit standpoint for monitoring tumor response during treatment. Circulating 'liquid biopsy' biomarkers have recently shown promise as metrics predictive of tumor immunotherapy response, because they can be non-invasively obtained from patients and trended over time (Figure 1) [6]. In this review, we begin by providing a brief overview of the FDA-approved checkpoint blockade immunotherapies, their mechanisms of actions, and the basic immunology of checkpoint inhibitors. We then explore and synthesize findings from studies published in the last five years identifying potential biomarkers predictive of clinical response for different cancer types and immunotherapies. We outline the major classes of

CONTACT Rajan P. Kulkarni kulkarnr@ohsu.edu 3303 SW Bond Ave, CH16D, Portland, OR, 97239

Article highlights

- Circulating 'liquid biopsy' biomarkers are promising non-invasive metrics for the prediction and tracking of treatment response to checkpoint blockade immunotherapy, and the highest quality evidence is available for metastatic melanoma and non-small cell lung cancer.
- Tumor PDL1 expression alone does not adequately capture the complexity of the host immunology and tumor microenvironment, and its predictive value seems to be limited to specific cancers or patient populations.
- Circulating tumor DNA, blood tumor mutational burden, transcriptomic signatures, circulating tumor cells, and host immunological markers are the most promising next-generation 'liquid biopsy' biomarkers with potential for translation into clinical practice.
- Selection and validation of biomarkers to predict tumor response to checkpoint blockade immunotherapies require cross-correlations between an individual's genetic background, tumor microenvironment, and immunological signatures.
- Integration of genomic and proteomic methods in concert with advancements in artificial intelligence and next-generation sequencing will enable cancer immunotherapy to transition toward personalized medicine.

potential biomarkers, highlight significant breakthroughs, identify key areas for improvement, and provide expert recommendations for how these diagnostic tools can be translated into clinical practice. Identification of promising biomarkers can potentially expedite the implementation of personalized medicine in cancer immunotherapy.

2. Overview of immunotherapies: drug classes and mechanisms of action

Checkpoint inhibitor immunotherapies are monoclonal antibodies directed at disrupting either the cytotoxic T lymphocyte-associated antigen 4 (CTLA4) pathway or the programmed cell death protein 1 pathway (PD1/PDL1). At present, the seven FDA-approved cancer immunotherapies are ipilimumab (CTLA4), nivolumab (PD1), pembrolizumab (PD1), atezolizumab (PDL1), avelumab (PDL1), durvalumab (PDL1), and cemiplimab (PD1) [7]. Ipilimumab is the only FDA-approved CTLA4-based therapy and was the first immunotherapy to market, while the most recently approved therapy was cemiplimab.

During normal T cell-mediated immune responses, T lymphocytes patrol the body for signs of infection, disease, or cancer. Before initiating a response, they first probe the target for cell surface markers, which may reveal its identity as healthy or unhealthy. Recognition of peptide antigens on unhealthy cells or on antigen-presenting cells (APCs) via the T cell receptor (TCR) leads to T cell activation and proliferation. T cells also normally express PD1 and CTLA4 on their surface, which are inhibitory receptors that prevent T cell activation when bound by ligands PDL1 or CD80/CD86, respectively [8,9]. Tumors may escape this type of surveillance by aberrantly expressing PDL1 or CD80/CD86, which activate PD1 or CTLA4, inducing inhibition of T cell activation and proliferation. The detailed immunology of CBI is reviewed thoroughly elsewhere [8].

The newest class of immunotherapies, chimeric antigen receptor (CAR) T cells, do not rely on checkpoint inhibition.

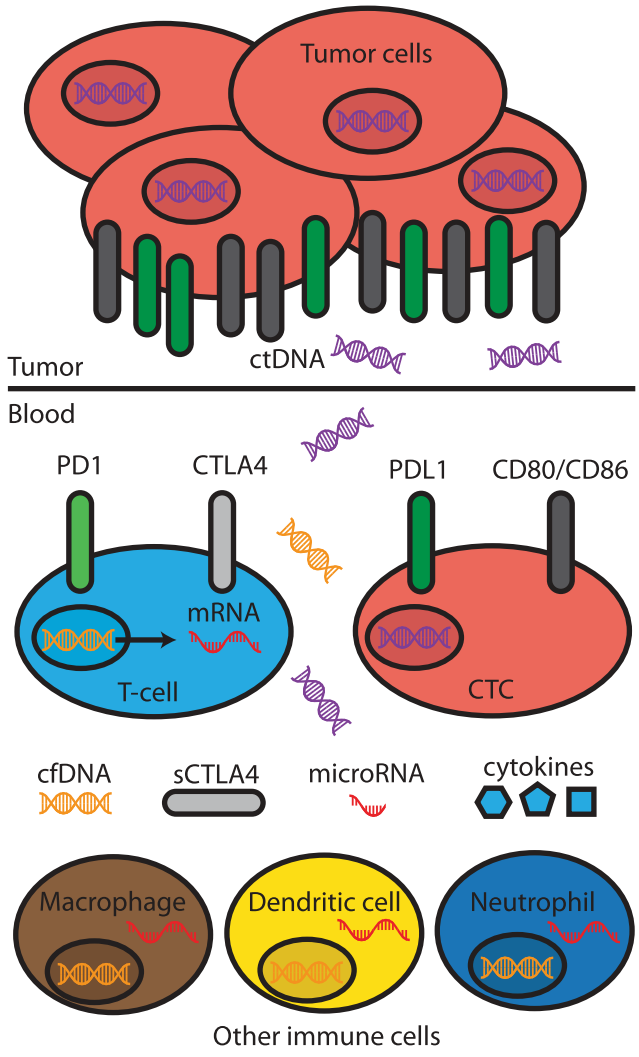

Figure 1. Overview of potential circulating 'liquid biopsy' biomarkers predictive of treatment response to checkpoint blockade immunotherapy. Genomic, transcriptomic, proteomic, and immunologic biomarkers are depicted.

Instead, they induce anti-tumor immunity by customizing the patient's T cells to recognize specific cell-surface markers on the target cancer. The process involves isolating the patient's own T cells, genetically engineering them to express CARs specific their tumor, and then injecting them back into the patient [10]. In this review, we will focus primarily on studies of cancer patients treated with checkpoint blockade immunotherapies.

3. Circulating markers predictive of response to immunotherapy

The majority of candidate biomarkers being vetted for prediction of treatment response can be categorized into genomic and proteomic markers. Examples of genomic studies include whole-exome sequencing circulating tumor cells [11], profiling of naked cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA) [12], and RNA transcriptomic signatures of host immune cells [13]. Proteomic markers include either soluble proteins present in circulation, such as host cytokines and chemokines [14], or cell-surface markers on circulating tumor or immune cells such as PD1/PDL1 and TCRs [9]. Another class

of soluble biomarkers that can predict response to immunotherapy are exosomes and extracellular vesicles [15,16]. Several studies have also proposed 'immune biomarkers' that measure the magnitude of the immune response generated with CBI, which are a direct reflection of host immunology and have been associated with response to treatment [17].

PDL1 expression was the first proposed proteomic biomarker for prediction of treatment response to CBI, since a number of studies noticed that patients undergoing CBI with tumors overexpressing PDL1 measured via immunohistochemistry had improved clinical outcomes. However, a large number of patients who had low levels of PDL1 also exhibited robust responses, which complicates the use of PD1/PDL1 as an exclusive biomarker [18]. While PDL1 expression has been associated with more favorable response rates to PD1/PDL1 agents, PDL1 is not a static biomarker capable of binary discrimination of responsiveness [19]. Furthermore, a number of clinical trials have measured PDL1 in patients, but the assessment methodology of PDL1 is heterogenous, making it different to compare reproducibility across trials [20]. Other proteomic and cell-based biomarkers are related to host immunology. For example, the neutrophil to lymphocyte ratio (NLR) has been found to be prognostic of survival in many solid tumor types. Interestingly, a recent meta-analysis showed that an NLR > 4 was associated with increased overall survival. Other studies have identified the absolute lymphocyte count (ALC) to be positively correlated with survival, and serum lactate dehydrogenase (LDH) to be correlated with a negative prognosis in patients with melanoma following ipilimumab therapy [21]. Other immunologic markers include the TCR, the inducible costimulatory (ICOS) molecule, and serum autoantibodies.. Additional potential biomarkers positively correlated with active response include mutated tumor antigens, cytokine signature indicative of CD8 activation, PDL1 expression, whereas those associated with no or limited response include high levels of immunosuppression [22].

3.1. Melanoma

Since the first FDA approval of ipilimumab, the community has searched for biomarkers predictive of response in melanoma, especially in specific genotypes (e.g. BRAF, NF1, NRAS) and subtypes of melanoma (e.g. desmoplastic, acral lentiginous melanoma). Emerging metrics to track responses to cancer immunotherapy in melanoma can be categorized broadly into genomic markers, proteomic markers, and immunologic markers [23]. The most studied markers include PDL1, ctDNA, transcriptomic signatures, and host immunologic markers, including absolute counts and ratios of immune cell subtypes, cytokines, chemokines, and other soluble proteins [24].

In a study of 49 melanoma patients, the molecular signature of microfluidically enriched circulating tumor cells (CTCs) were analyzed using a quantitative 19-gene digital RNA signature (CTC score) [25]. Patients with high quantities of CTCs had a significantly higher risk of relapse, whereas those with decreasing or stable numbers had longer overall survival, and a decrease in CTC score within 7 weeks of CBI correlated with an increase in progression-free survival (hazard ratio (HR), 0.17; P = 0.008) and overall survival (HR, 0.12; P = 0.04) [13]. Tumor-specific mutations in ctDNA such as BRAF and NRAS mutations for melanoma patients have been proposed for monitoring of immunotherapy response [26]. In a review of seven cases, a recent study showed that comprehensive BRAF/NRAS ctDNA monitoring during anti-PD1 can be used during anti-PD1 treatment to monitor clinical benefit [27], which agrees from findings in another study of 229 patients [28]. In a study of 35 patients with combined CTLA4 and PD1 blockade therapy, 710 tumor-associated genes were studied from repeated liquid biopsies before and during treatment. TMB obtained from ctDNA was higher in responders than non-responders with a cutoff of TMB > 23.1. Furthermore, a decrease in over 50% of TMB after the first 3 weeks of treatment led to increased overall survival [29].

Another emerging class of biomarkers are microRNAs (miRNAs), which are released dynamically from dying tumor cells. One of the first miRNAs shown to predict CBI responses was circulating miRNA-21 [30]. Since then, other studies have surveyed larger panels of microRNAs in circulating blood. Interestingly, in one study, several tumor-derived microRNAs were found to induce myeloid suppressor cells and predict immunotherapy resistance in melanoma and poor survival (miR-146a, miR-155, miR-125b, miR-100, let-7e, miR-125a, miR-146b, miR-99b) [31]. Circulating tumor DNA is another emerging biomarker to monitor treatment response during CBI in melanoma [32,33]. In a study of 86 patients, ctDNA was collected and correlated with stage and outcome, and found to be an accurate predictor of tumor response. Conversely, elevated ctDNA after therapy correlated with a poor prognosis [34]. Another study extended this work by using droplet digital PCR (ddPCR) to study ctDNA post-therapy as opposed to pre-therapy or during therapy. ddPCR data showed that ctDNA levels fell upon treatment response and rose with detectable disease progression, and was superior to LDH as a blood-based marker [35].

Host immune cell-derived biomarkers such as serum immunoregulatory proteins also have emerged. A great deal of work has been done to identify signatures of strong immune host responses, which are thought to correlate with increased probability of therapy response. Soluble CTLA4 (sCTLA4) was explored as a possible biomarker for identifying a subset of patients that respond to ipilimumab therapy. In 113 patients, high sCTLA4 serum levels predicted favorable clinical outcomes with ipilimumab treatment [36]. In contrast, high baseline levels of soluble CD25 (sCD25) are associated with a poor prognosis and treatment resistance with CTLA4 blockade [37]. Another study of 194 patients showed that low levels of soluble NKG2D ligands MICB, ULBP1, and ULBP2 were associated with clinical outcomes in CBI [38]. LDH was one of the first biomarkers to make it into clinical guidelines as an independent predictor of survival in melanoma [39]. In one study of 209 patients, a baseline signature of low LDH, absolute monocyte counts (AMC), and myeloid-derived suppressor cells (MDSCs) as well as high absolute eosinophil counts (AEC), regulatory T-cells (Tregs), and relative lymphocyte counts (RLC) are associated with favorable outcome following ipilimumab [40]. In a cognate study, LDH levels significantly increased over baseline (10–40%) was associated with

significantly shorter survival times [41]. Similarly, elevated levels of IL-15, TIM-3, and NK cell subsets predict responsiveness to anti-CTLA4 treatment in melanoma [42]. Circulating IL-17, TGF-β1, and IL-10 are predictors of response in ipilimumab neoadjuvant therapy of melanoma. In 35 patients, IL-17 was associated with toxicity/side effects, while TGF-β1 and IL-10 led to improvement in therapeutic clinical outcome with respect to progress-free survival [43]. In 273 patients receiving CTLA4 blockade, elevated levels of chemokine CXCL11 and soluble MHC class I polypeptide-related chain A (sMICA) were linked to poor overall survival [44].

In a separate study looking at cell-surface markers on host immune cells, the authors leveraged advances in high-through mass cytometry to conduct high-dimensional single-cell analysis utilizing a machine learning-based pipeline for characterization of diverse immune cell subsets in the peripheral blood of patients with stage IV melanoma before and after 12 weeks of anti-PD1 immunotherapy. They found that the strongest predictor of progression-free and overall survival was the presence of CD14+ CD16− HLA-DRhi monocytes in response to CBI [45]. Similarly, another trial found that increases in ALC and circulating CD4 and CD8 T cells are linked to increased positive clinical outcomes with ipilimumab treatment [46]. An increase in total circulating lymphocytes [47] and decreased NLR [48] were also associated with higher survival. Similarly, elevated levels of CD16-expressing monocytes at baseline were associated with higher response rates [49]. Conversely, in another study of 720 patients with advanced melanoma, those with both absolute neutrophil counts (ANC) ≥ 7500 and NLR ≥ 3 had a significantly increased risk of death due to decreased response [50]. An increased diversity of TCRs and T cell repertoire are both associated with a favorable response to CTLA4 blockade [51,52]. In concordance with these findings, both PDL1 expression on peripheral circulating T cells and the presence of CD137 on circulating CD8 T cells was associated with better prognosis with respect to overall and progression-free survival [53]. Gene expression profiles in host immune cells were also explored as potential markers. Expression of genes involved in cytolytic activity and proliferation of NK cells and T cells were related to positive responses in both anti-CTLA4 and anti-PD1 therapies [54]. In a subset of patients with melanomas expressing the NY-ESO-1 tumor antigen, the presence of anti-NY-ESO-1 antibodies along with corresponding CD8 T cells experienced more frequency clinical benefit with ipilimumab [55].

A newer class of biomarker is represented by exosomes and extracellular vesicles, which are released from tumors into the circulation. A study of patients with metastatic melanoma showed that exosomes released from melanomas carry PDL1 on their surface, and that the increase in levels of circulating exosomal PDL1 correlates with tumor response to anti-PD1 therapy, and also tracks with IFN-γ stimulation [56]. Suppression of exosomal PDL1 was found to induce systemic anti-tumor immunity and promotes T cell activity in the draining tumor lymph node, suggesting that exosomal PDL1 could be a therapeutic target in metastatic melanoma [57]. Interestingly, measurement of mRNA levels of exosomal PDL1 by PCR rather than direct measurement of PDL1 proteins within circulating exosomes is sufficiently predictive [58].

3.2. Non-small cell lung cancer (NSCLC)

Currently, PDL1 is the only biomarker used in clinical practice to select patients most likely to benefit from CBI in NSCLC. Conflicting findings have been associated with PDL1 as a biomarker in NSCLC. In the largest study, 2102 patients who underwent nivolumab therapy were studied. For those with PDL1 expression of <1% via immunohistochemistry (IHC), nivolumab showed a trend for improved survival compared with docetaxel. Although PDL1 expression is related to greater response, PDL1 negative patients had also some benefit [59]. In another study of 914 patients, pooled analysis showed that patients with PDL1 positive tumors had a significantly higher overall response rate, compared to patients with PDL1 negative tumors (OR: 2.44; 95% CIs: 1.61–3.68). They suggested an IHC cutoff point of 1% for positivity as a predictive biomarker for the selection of patients to treat with immune-checkpoint inhibitors [60]. CTC counts were one of the early markers proposed for therapy responses in primary lung cancer [61]. In a study of 24 stage 4 NSCLC patients treated with nivolumab, CTCs were analyzed for PDL1 expression. Interestingly, patients with PDL1 negative CTCs all obtained a clinical benefit, while patients with PDL1 positive CTCs all experienced progressive disease [62]. In another study using an engineered microfluidic system to isolate and concentrate NSCLC CTCs, PDL1 expression on CTCs alone was not predictive of progression-free survival [63]. These findings have triggered research into identification of other biomarkers that are more reliable for predicting response to CBI. Within the last several years, genomic and host immunology-based markers borrowed from the melanoma literature have been proposed and studied, such as TMB, tumor microenvironment, and immune cell-related biomarkers.

A key theme is that the genomic landscape of lung cancers shape responses to anti-PD1 therapy. Recent data show that TMB obtained from peripheral sampling of ctDNA in the blood is one likely candidate ready to enter clinical practice to aid treatment selection [64]. TMB from tumor samples, as measured by next-generation sequencing (NGS) [65], whole-exome sequencing (WES) or a cancer gene panel (CGP) [66], is known to be associated with immunotherapy responses. However, whether TMB estimated by ctDNA in circulating blood (bTMB) is associated with clinical outcomes of immunotherapy remains to be explored. In a groundbreaking study, a CGP named NCC-GP150 was designed and virtually validated using a large-scale patient database based on blood samples. In 50 patients, they found that the bTMB estimated by NCC-GP150 distinguished between patients that would or would not benefit from CBI, and validating the bTMB as a useful prognostic biomarker [11]. In a study of 136 patients with NSCLC, a higher ctDNA TMB was significantly correlated with poor clinical outcomes with CBI [67], which is interestingly in contrast with a separate study of TMB in direct tumor samples, which showed a higher mutational burden in ctDNA was associated with improved overall survival and progress-free survival [68]. This suggests that ctDNA may reflect a different genomic signature than the tumor DNA. In a later study, blood-based TMB predicted clinical benefit in NSCLC to atezolizumab, which was validated with a retrospective analysis of

two large randomized trials [69]. Recently, a plasma immune-related miRNA-signature classifier (MSC) that is typically used in screening patients for lung cancer was applied to determine whether it was also predictive of response to CBI in NSCLC [70]. They found that the MSC test was associated with overall and progression-free survival, and that the MSC and PDL1 combination panel of biomarkers were able to risk-stratify patients into three groups. Furthermore, the MSC risk level remained low in patients until tumor progression was measured, suggesting that it can be used to track recurrence. Another distinct study proposed that ctDNA levels could enable early assessment of immunotherapy efficacy. A drop in ctDNA level is an early marker of therapeutic efficacy and predicts prolonged survival in patients treated with immune-checkpoint inhibitors for NSCLC [71]. Very early response of circulating tumor-derived DNA in plasma predicts efficacy of nivolumab treatment in patients with non-small cell lung cancer. Fourteen patients who were treated with nivolumab. Levels of ctDNA measured, basal and serial ctDNA analysis revealed that a decrease in allelic frequency (AF) of ctDNA showed high-level correspondence with a good durable response at the 2-week mark [72]. Overall, ctDNA represents an extremely promising biomarker in NSCLC.

Similar to metastatic melanoma, immunologic host-related biomarkers are now being explored as potential predictors of response to CBI. Examples of these immunologic host-related biomarkers reflective of immunotherapy response in NSCLC include ALC [73], ANC [73], NLR [74], platelet to lymphocyte ratio (PLR) [75], AEC [73], and CD8 T-cell density [76]. These markers have demonstrated prognostic value in small studies. In a recent study, NLR is correlated with survival in patients treated with PD1/PDL1 blockade, where low NLR Is associated with better outcomes. NSCLC patients undergoing PD1/PDL1 blockade showed that reduction in NLR during treatment is correlated with treatment response using computed tomography imaging as an endpoint, with progressive disease corresponding to an increase in the NLR [77]. In a study of 70 treatment naïve NSCLC patients, decreased survival was associated with elevated levels PD1+, PD1+ CD3+, PDL1+ CD3+, PDL1+ CD3+ CD8+, PDL2+ CD3+, PDL2+ CD3+ CD4+, or PDL2+ CD3+ CD8+ PBMCs. Interestingly, the cytokines IL-2 and TNF-α were strongly associated with the expression of PDL1 on T cells in responding patients [78].

4. Other cancers and cancer invariant 'universal biomarkers'

Compared to metastatic melanoma and NSCLC, fewer studies have been conducted on identifying novel biomarkers for urothelial carcinoma [4], head and neck cancers [79], colorectal cancer [80], and breast cancer [81]. Here, we summarize the results of a subset of these studies. Potential biomarkers that have been identified in genitourinary malignancies include mutational burden, PDL1, cytokine panels, and autoimmune responses like vitiligo, colitis, and thyroiditis [82]. In urothelial carcinoma, a recent study identified alterations in DNA damage and repair (DDR) genes and mutational load as correlated with improved clinical outcomes after PD1/PDL1 blockade. Sixty patients with urothelial cancer enrolled in prospective trials of anti-PD1/PDL1 antibodies met inclusion criteria. DDR alterations are independently associated with response to PD1/PDL1 blockade in patients with metastatic urothelial carcinoma [4]. In head and neck cancers, anti-PD1 agents have become the standard of care for platinum-refractory recurrent/metastatic head and neck squamous cell carcinoma (HNSCC). A recent study showed that a combination of PDL1 expression and circulating CD8 T cells have positive predictive value [83]. In 113 patients with HNSCC, detection of CTCs overexpressing PDL1 was found to have prognostic value in HNSCC. Overexpression of PDL1 at end of treatment had poor survival compared to those without, and the abscess of PDL1 overexpression at end of treatment was associated with complete response [79]. In 25 patients with muscle invasive and metastatic bladder cancers, PDL1 was characterized on CTCs, which could potentially guide treatment selection [84]. Historically, response to CBI in colorectal carcinoma has been poor but a small subset of patients do respond to CBI. In 50 patients with metastatic colorectal carcinoma, a panel of six CTC markers were measured (GAPDH, VIL1, CLU, TIMP1, LOXL3, and ZEB2) and correlated with overall and progress-free survival. Reduction in these six CTC markers corresponded to a doubling in both outcomes, compared to those with high CTC markers. Interestingly, treatment-refractory patients could be identified using the same panel that were misidentified as responders via computed tomography imaging [80]. Circulating levels of PDL1 present on exosomes, but not freely circulating PDL1, released from head and neck cancers were associated with disease progression, and blockade of PDL1 exosome signaling correlated with a robust immune response [85]. Exosomes may also represent early biomarkers for ovarian cancer [86].

Most studies thus far have assessed biomarkers predictive of response in specific cancer types, but recent work has expanded the idea of cancer invariant 'universal biomarkers' that are indicative of pan-tumor responses to CBI. In a key study, other proteomic markers such as CTLA4 expression and the absence of the cytokine fractalkine (CX3CL1) were also associated with strong immune responses [87] across multiple cancer types. Other cytokines or chemokines prognostic of positive response included increase levels of IFN-γ and IL-18, and decreased levels of IL-6. Transient increases in CD8+ HLA-DR+ Ki-67+ lymphocytes were associated with CBI response in bladder cancer and other cancers [87,88]. In advanced solid tumor patients, CTCs were analyzed for PDL1 expression. PDL1 positive CTC and PDL1 high CTC correlate with disease outcome ($P < 0.001$, $P = 0.002$, and $P = 0.007$, respectively), and an abundance of PDL1 CTCs at baseline before treatment were predictive of progression-free survival [89]. Using next-generation sequencing from plasma/serum–derived cfDNA, another study quantified chromosomal instability across multiple cancer types. They identified that cfDNA could be used as a real-time surrogate for disease progression, as well as an early indicator of response to immunotherapy [12]. In NSCLC, uveal melanoma, or colorectal cancer patients treated with nivolumab or pembrolizumab monotherapy, changes in ctDNA levels during therapy could be a promising tool for very accurate monitoring of treatment efficacy [90]. They found that patients with undetectable ctDNA at 8 weeks responded well to therapy, and predicted higher PFS and overall survival [91].

5. Conclusions

In this review, we began by briefly outlining the FDA-approved checkpoint blockade immunotherapies and their mechanisms of actions. We summarized key findings from primary studies published in the last five years identifying potential metrics predictive of clinical response, with a focus on biomarkers for metastatic melanoma, NSCLC, and 'cancer invariant' biomarkers. We outline the major classes of potential biomarkers, which can be divided broadly into genomic signatures and proteomic signatures. Although many candidate biomarkers have been described to date, only three assays are FDA-approved (one as a companion and two as a complementary diagnostic [92]) to identify patients who are more likely to benefit from anti-PD1 /PDL1 therapies [93]. We discussed advancements in biomarker identification and validation utilizing multimodal approaches such as deep sequencing, transcriptomics, and machine learning. A number of studies identified combinations of genomic and proteomic biomarkers that were not necessarily predictive of immune response by themselves but were strongly correlated with survival when considered in combination [94]. As a result, multiplexed detecting methods and biomarker panels may provide new strategies for addressing the question of predicting therapy response. Several studies have identified circulating tumor DNA [95] and tumor mutational burden as prognostic, in addition to some oncogene mutations. Circulating proteomic markers like cytokines/chemokines and the numbers of or ratios of specific tumor-tropic immune cells, such as neutrophils, CD4 T cells, and CD8 T cells are of high predictive value as well. As current evidence of those potential predictors, a consensus and standardization is required to apply these biomarkers broadly to larger patient populations [96]. The most promising biomarker strategies beyond PD1/PDL1 encompass genomic analysis of circulating tumor DNA and cell-free DNA (microsatellite instability, specific tumor mutations, DNA damage), the tumor mutational landscape, and proteomic and transcriptomic signatures of host immunology [97]. Future development of predictive biomarkers for CBI must integrate multiple approaches to characterize host immunology and tumor immunology [98].

The highest quality evidence at the present moment is available for metastatic melanoma and non-small cell lung cancer. Further studies will need to be conducted for other cancer types, including urothelial carcinoma, colorectal carcinoma, and renal cell carcinoma. Although we focused on biomarkers predictive of tumor response to checkpoint inhibitor immunotherapies, other immunotherapy modalities are being readily explored in both basic research and clinical trials. One example is CAR T cell immunotherapy, which has been recently PDA approved for lymphoma. At present, there are no studies identifying circulating biomarkers predictive of tumor response to CAR T cell immunotherapy, and more work will need to be done to identify such biomarkers. In the broader class of non-invasive biomarkers, several recent studies have identified multimodal-targeted imaging-based biomarkers for tumor response, termed 'radiomics' [99,100]. For example, positron emission tomography (PET) with the development of new tracers specific for various cancers can enable another non-invasive and quantitative strategy to monitor treatment response [6].

6. Expert opinion

Selecting an optimal panel of biomarkers that are predictive of response to tumor immunotherapy is confounded by numerous factors, including but not limited to patient-to-patient heterogeneity, tumor genetic heterogeneity, sensitivity and specificity of diagnostic tests, costs, and regulatory considerations [93]. The first biomarkers proposed to predict treatment response to CBI were based on PD1 and PDL1 expression on tissue sections, but their predictive value seems to be limited when evaluated in a vacuum, since some patients with PDL1 negative tumors retain robust immune responses, while in other cancers, it does not correlate with treatment response at all. Dissecting how and why this occurs from an immunologic standpoint is currently under investigation. At present, there is no consensus or standardization of approaches for identifying and validating potential biomarkers. Right now, the main barriers to clinical adoption are two-fold: selecting and validating biomarkers for specific patient populations using a standardized procedure [93,101] and translating novel findings from individual smaller studies toward broad applicability in larger patient populations. Some work has been done to standardize approaches to identifying biomarkers. Recently, the Society for Immunotherapy of Cancer convened the Immune Biomarkers Task Force, consisting of a multidisciplinary panel of experts to make recommendations [102]. Addressing these problems will require both advancement of our basic science knowledge of how CBI works, specifically how administration affects both host and tumor genetics and immunology, as well as clinical testing of potential markers with real-world patient data.

Common limitations of some present studies include typical statistical limitations such as deficiencies in sample size and power, which can be easily rectified. A very important feature of well-validated biomarkers that most likely will be implemented in the clinic is that they must possess a strong negative predictive value, which do not limit patients with falsely negative results from receiving benefit from CBI [23]. This should be kept in mind during the selection of biomarkers. Other considerations for biomarker development include their use an adjunct to guide selection of medications with unfavorable risk–benefit balance, especially those with severe side effects. Identifying 'hidden responders' in a haystack of mostly non-responders may uncover new biomarkers that are indicative of response. Conversely, those that are not necessarily predictive of response can still identify patients that can respond to therapy, as evidenced by PD1/PDL1 [103]. More importantly, proper clinical trial design and implementation of biomarker monitoring before and during treatment will be central to collecting high-quality data patient data [104].

The studies discussed in this review outline not only potential new biomarkers for prediction of response to CBI but also illuminate new tools and technologies for selecting optimal biomarkers from a pool of candidates. Lessons learned from other major fields, such as computer science and biomedical engineering can be applied effectively to oncology. For example, engineered microfluidic devices can assist in capturing circulating tumor cells for molecular characterization [63], while machine learning approaches can help identify

immunological signatures predictive of responses [45]. Due to increased interest in exosomes and extracellular vesicles containing PDL1 as circulating biomarkers, state of the art methods aimed at isolating and purifying exosomes from varying bodily fluids has been developed [105]. Due to the fast-growing nature of the field, we believe that changes can be realistically implemented into clinical and research practice. However, this will require a multidisciplinary approach, involving collaborations between surgeons, oncologists, immunologists, bioinformaticians, computer scientists, and regulatory bodies across multiple institutions. We believe that technical and technological limitations lie primarily in the novel application of existing technologies, rather than the lack of developed technology, and that the CBI field will benefit immensely from cross-disciplinary assimilation of ideas.

We anticipate that in the next 5–10 years, integration of genomic and proteomic methods in concert with advancements in artificial intelligence and next-generation sequencing will enable cancer immunotherapy to transition toward personalized medicine [21,106,107]. With numerous ongoing clinical trials testing new and existing CBIs, there will be a wealth of data moving forward that can be efficiently mined and analyzed using bioinformatics [108]. We envision that efficient selection and validation of biomarkers to predict tumor response to CBI will require cross-correlations between an individual's genetic background, tumor microenvironment, and immunological signatures. Our hope is that a number of biomarker panels will become FDA approved for screening patients. We also anticipate that new combination drug regimens [109] as well as new modalities such as CAR T-cell therapy [10] and cancer vaccines [110] will prove useful in further improving overall survival and progression-free survival, and that new biomarkers will need to be identified to track treatment responses for these therapies.

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