Biomarkers for assessing the effectiveness of immunotherapy in breast cancer

Melissa J Nixon¹ & Justin M Balko^{*,1}

¹Vanderbilt University Medical Center, Vanderbilt University, Nashville, TN 37232-6307, USA

* Author for correspondence: justin.balko@vanderbilt.edu

“PD-L1 continues to be the most common biomarker assessed across cancer types, but a standardized protocol needs to be developed to facilitate data interpretation across clinical trials.”

First draft submitted: 22 November 2017; Accepted for publication: 23 November 2017; Published online: 17 January 2018

Keywords: biomarker • breast cancer • CTLA-4 • immune checkpoint blockade • immunotherapy • mutation burden • neoantigen • PD-L1 • triple-negative breast cancer • tumor-infiltrating lymphocytes

The fundamental role of the immune system is to maintain tissue homeostasis through continued surveillance of tissues. Normal tissue function can be altered by the presence of a tumor which induces an immune response. Through continued immunoediting, tumors can evolve mechanisms to circumvent immune recognition. When the immune system is activated against an insult, the resulting cytotoxic responses must be fine-tuned to prevent damage to surrounding tissue. One such homeostatic mechanism in immune cells is to upregulate immune checkpoint molecules, such as PD-1 and CTLA-4. Engagement of these receptors with their partners on the surface of other cells (PD-L1/PD-L2 for PD-1 and CD80/CD86 for CTLA-4) attenuates signals within the T-cell preventing effector function. Thus, a therapeutically tractable mechanism of immune evasion is through induction of these inhibitory ligands which engage their cognate inhibitory checkpoints on immune cells to suppress function.

These interactions can be targeted with monoclonal antibodies known as immune checkpoint inhibitors (ICIs). ICIs function by blocking the inhibitory receptor/ligand interaction and reinvigorating antitumor effector activity. ICI therapy may also promote immune memory, leading to durable responses. The first clinical success in this area was observed with ipilimumab, a CTLA-4 inhibitor that drastically improved the survival of a fraction of metastatic melanoma patients in combination with dacarbazine [1]. Subsequently, other ICIs, such as pembrolizumab (anti-PD-1) and nivolumab (anti-PD-1), have transformed the standard of care for metastatic melanoma patients and improved the 1-year survival rate from 35% to up to 70% [2]. This has led to US FDA approval of ICIs in melanoma and non-small-cell lung cancer. Clinical trials evaluating ICIs are currently ongoing in breast cancer, with a focus on metastatic triple-negative breast cancer (TNBC), as these patients have the lowest median overall survival (OS; less than 1 year) [3].

TNBC patients have been proposed to be candidates for ICIs due to the abundance of tumor-infiltrating lymphocytes (TILs) relative to other breast cancer subtypes. In TNBC, TILs can predict response to chemotherapy and prognosticate OS, suggesting an active immune component to the disease [4–6]. Concordantly, TNBCs have the highest genomic instability and higher rates of genetic mutations compared with other breast cancer subtypes, putatively producing more neoantigens and increased immunogenic potential [7]. So far, eight studies have been published in heavily pretreated breast cancer patients with PD-1/PD-L1 inhibitors, which yielded response rates from 5 to 19% with one study achieving an overall response rate of 42%, with some responses lasting more than a year [8–15]. The overall low response rates to these agents suggest that suitable biomarkers of response will be critical to their success by identifying patients that may achieve the greatest benefit from these costly and potentially toxic agents.

However, it is important to consider that a substantial amount of work has already been performed in other tumor types in attempt to identify clinically utilizable biomarkers of ICI response. Furthermore, while many of these biomarkers are capable of statistically predicting ICI response, there are a number of challenges associated

with their clinical deployment. These challenges are widespread and include costly or computationally intensive assays, high degree of variability in clinical interpretation, heterogeneity in predictive power across datasets and insufficient positive or negative predictive values for routine clinical utility, among others. Several of these established biomarkers, their challenges and their potential for utility in breast cancer are described below.

TIL identification

Diagnosis of TNBC occurs through diagnostic pathology. Hematoxylin and eosin (H&E) stained slides from the tumor are used as a low cost assay routinely used to assess clinical parameters. TILs can be identified based on morphology in an H&E slide allowing for seamless integration into standard diagnostic practices in breast cancer. In melanoma, an increase in TILs between baseline and 3 weeks after ipilimumab (anti-CTLA-4) correlated with clinical response [16]. In breast cancer, TILs in the stromal compartment predicted response to neoadjuvant chemotherapy [6]. Additionally, in breast cancer patients who had residual disease after neoadjuvant chemotherapy, high TIL infiltrate predicted better OS compared with those with low TIL infiltrate [4], suggesting that antitumor immunity can modulate outcome. Concordantly, in an early trial conducted with atezolizumab in metastatic TNBC, patients with high TILs had a 6 month increase in OS compared with low TIL burden, suggesting that TILs may be a biomarker of response to NAC and ICI [15]. One limitation to the assessment of TILs by H&E is the nonspecific nature of the assay; this method cannot quantify specific immune subsets, which could provide insight into their antitumor activity. Interestingly, there was a strong correlation, however, between TIL and CD8⁺ staining in matched patient samples, suggesting that H&E TILs assessment might be a reasonable surrogate for effector T cells [6].

PD-L1 IHC expression

PD-L1 expression, as assessed by immunohistochemistry (IHC), is perhaps the most commonly examined biomarker for response to therapies targeting the PD-1/L1 axis. The highest rates of PD-L1 expression (20–30%) are found in TNBC patients compared with other breast cancers [17]. Several studies have shown that PD-L1 expression provides a favorable outcome with ICI, but patients that are PD-L1 negative by IHC also demonstrate durable responses in some cases. In a prospective analysis of patients with metastatic BRAF-wild-type melanoma treated in a Phase III clinical trial with nivolumab, PD-L1⁺ cells in at least 5% of the tumor did not show a difference in outcome for these patients [2]. Similarly, 81% of PD-L1-positive TNBC patients were completely refractory to atezolizumab in a Phase Ia study [8]. In addition to PD-L1 being a difficult antigen to detect after fixation, PD-L1 expression is highly dynamic and expression can change in response to different stimuli and microenvironments (e.g., IFN- γ), which may influence correlation with response. In the eight ICI trials in TNBC with reported results, patients positive for PD-L1 expression on either tumor cells or immune cells ranged from 19.4% to 68% using expression cutoff rates ranging between >1 and >5%, and using either the 22C3 or SP142 PD-L1 antibody clone [8–15]. Overall response rates ranged from 5 to 42% and PD-L1 expression failed to predict benefit from ICI in any study. These data suggest that PD-L1 may not be a sufficiently reliable clinical assay to be used as a biomarker to exclude patients from receiving ICI, given the potential for durable responses in PD-L1 negative patients. In addition, there are inconsistencies in the antibody clones, cell types scored and positivity cutoffs to make broad determinations in its clinical utility. Thus, in optimal settings, PD-L1 expression by IHC might enrich for responders, but is clearly insufficient as a stand-alone assay.

Mutation burden

Cancer is driven by genomic alterations that can create novel protein products recognized by the immune system as neoantigens. As clinical trials are performed with eligibility criteria based on somatic alterations within the tumor such as I-SPY, I-SPY2 and NCI-MATCH, the tools to determine somatic mutations are becoming more accessible and widely used. Mutation burden assessed by whole exome sequencing or targeted next generation sequencing (NGS) might be used to estimate the tumor neoantigen repertoire available for identification by patrolling T cells. In non-small-cell lung cancer, patients with >200 mutations derived greater benefit from PD-1 ICI [18]. Additionally, in melanoma using a smaller NGS panel, patients who responded to anti-PD-1 or anti-PDL1 had higher mutational loads than nonresponders (median 45.6 vs 3.9 mutations/megabase) [19]. In melanoma, however, bioinformatic analysis of patients in The Cancer Genome Atlas suggests that neoantigen and tumor mutation burden are not directly associated with T cell gene expression signatures in the microenvironment [20]. Therefore, it is not currently clear if mutational analysis can provide additional or independent information to T cell or lymphocyte quantitation

in predicting ICI response. While NGS analysis is computationally intensive, it is becoming more affordable and less cumbersome with established data analysis pipelines. Furthermore, it is commonly employed in the clinic for the identification of targetable oncogenes. Nonetheless, mutational analysis does not directly sample or provide molecular information on the immune microenvironment, which is likely an important independent factor in ICI outcome.

Conclusion

The development of biomarkers of response associated with ICI is needed to enrich for patients more likely to have durable responses to ICI with limited toxicities. The utility of these biomarkers must be better studied through standardized and independently validated assays in larger prospective studies of patients undergoing ICI. PD-L1 continues to be the most common biomarker assessed across cancer types, but a standardized protocol needs to be developed to facilitate data interpretation across clinical trials. Furthermore, PD-L1 IHC has routinely failed to demonstrate consistent or clinically sufficient predictive power to be more than a temporary stand-alone biomarker. TIL assessment may be a useful biomarker that can be easily obtained with little cost and can be implemented in multivariate predictors, but it likely lacks the predictive power to be a stand alone assay. However, appropriate and standardized quantitation methods may help improve its utility. As a field, it is critical that the correlative analyses of ongoing breast cancer trials not only employ the most reproducible and standardized methods for previously identified biomarker assays (e.g., PD-L1 and TILs), but also break new ground by using novel biologically supported assays for discovery of improved biomarkers of ICI benefit in breast cancer.

Financial & competing interests disclosure

This article was supported by the NIH (grant no. 6R00CA181491). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

- 1 Robert C, Thomas L, Bondarenko I *et al.* Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N. Engl. J. Med.* 364(26), 2517–2526 (2011).
- 2 Robert C, Long GV, Brady B *et al.* Nivolumab in previously untreated melanoma without *BRAF* mutation. *N. Engl. J. Med.* 372(4), 320–330 (2015).
- 3 Den Brok WD, Speers CH, Gondara L, Baxter E, Tyldesley SK, Lohrisch CA. Survival with metastatic breast cancer based on initial presentation, de novo versus relapsed. *Breast Cancer Res. Treat.* 161(3), 549–556 (2017).
- 4 Loi S, Dushyanthen S, Beavis PA *et al.* RAS/MAPK activation is associated with reduced tumor-infiltrating lymphocytes in triple-negative breast cancer: therapeutic cooperation between MEK and PD-1/PD-L1 immune checkpoint inhibitors. *Clin. Cancer Res.* 22(6), 1499–1509 (2015).
- 5 Adams S, Gray RJ, Demaria S *et al.* Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two Phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. *J. Clin. Oncol.* 32(27), 2959–2966 (2014).
- 6 Denkert C, von Minckwitz G, Brase JC *et al.* Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. *J. Clin. Oncol.* 33(9), 983–991 (2015).
- 7 Banerji S, Cibulskis K, Rangel-Escareno C *et al.* Sequence analysis of mutations and translocations across breast cancer subtypes. *Nature* 486(7403), 405–409 (2012).
- 8 Emens LA, Braiteh FS, Cassier P *et al.* Abstract 2859: inhibition of PD-L1 by MPDL3280A leads to clinical activity in patients with metastatic triple-negative breast cancer (TNBC). *Cancer Res.* 75(15 Suppl.), 2859 (2015).
- 9 Nanda R, Chow LQ, Dees EC *et al.* Abstract S1-09: a Phase Ib study of pembrolizumab (MK-3475) in patients with advanced triple-negative breast cancer. *Cancer Res.* 75(9 Suppl.), S1-09 (2015).
- 10 Adams S, Diamond JR, Hamilton EP *et al.* Phase Ib trial of atezolizumab in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer (mTNBC). *J. Clin. Oncol.* 34(15 Suppl.), 1009–1009 (2016).
- 11 Rugo H, Delord J-P, Im S-A *et al.* Abstract S5-07: preliminary efficacy and safety of pembrolizumab (MK-3475) in patients with PD-L1-positive, estrogen receptor-positive (ER+)/HER2-negative advanced breast cancer enrolled in KEYNOTE-028. *Cancer Res.* 76(4 Suppl.), S5-07 (2016).
- 12 Dirix LY, Takacs I, Jerusalem G *et al.* Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a Phase Ib JAVELIN Solid Tumor study. *Breast Cancer Res. Treat.* doi:10.1007/s10549-017-4537-5 (2017) (Epub ahead of print).

- 13 Nanda R, Liu MC, Yau C *et al.* Pembrolizumab plus standard neoadjuvant therapy for high-risk breast cancer (BC): results from I-SPY 2. *J. Clin. Oncol.* 35(15 Suppl.), 506 (2017).
- 14 Adams S, Schmid P, Rugo HS *et al.* Phase 2 study of pembrolizumab (pembro) monotherapy for previously treated metastatic triple-negative breast cancer (mTNBC): KEYNOTE-086 cohort A. *J. Clin. Oncol.* 35(15 Suppl.), 1008 (2017).
- 15 Schmid P, Cruz C, Braiteh FS *et al.* Atezolizumab in metastatic TNBC (mTNBC): long-term clinical outcomes and biomarker analyses. *Cancer Res.* 77(13 Suppl.), Abstract 2986 (2017).
- 16 Hamid O, Schmidt H, Nissan A *et al.* A prospective Phase II trial exploring the association between tumor microenvironment biomarkers and clinical activity of ipilimumab in advanced melanoma. *J. Transl. Med.* 9, 204 (2011).
- 17 Mittendorf EA, Philips AV, Meric-Bernstam F *et al.* PD-L1 expression in triple-negative breast cancer. *Cancer Immunol. Res.* 2(4), 361–370 (2014).
- 18 Rizvi NA, Hellmann MD, Snyder A *et al.* Cancer immunology: mutational landscape determines sensitivity to PD-1 blockade in non-small-cell lung cancer. *Science* 348(6230), 124–128 (2015).
- 19 Johnson DB, Frampton GM, Rioth MJ *et al.* Targeted next generation sequencing identifies markers of response to PD-1 blockade. *Cancer Immunol. Res.* 4(11), 959–967 (2016).
- 20 Spranger S, Luke JJ, Bao R *et al.* Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. *Proc. Natl Acad. Sci. USA* 113(48), E7759–E7768 (2016).