At the crossroad between checkpoint blockade and big data analyses: identification of novel biomarkers and potential targets

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Editorial

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A tremendous step in immune-oncology was represented by the discovery of immune checkpoints (ICs) involved in the regulation of anti-tumor immune response. Currently, two important ICs are being recurrently discussed in oncology, PD-1 and CTLA-4 [1]. In 2011, the first successful IC inhibitor (ICI), ipilimumab, was approved for the treatment of metastatic melanoma [2]. In the following years, we witnessed a rapid expansion of cancer immunotherapeutics which offered a wide use of ICIs (anti-PD-1/PD-L1) in several malignancies [3], better insights in the role of tumor-infiltrating lymphocyte (TIL) [4], and the development of chimeric antigen receptor (CAR) T cells [5]. ICIs are the first class of therapeutics that have been approved not only for individual cancer indications, but also for solid malignancies presenting with high tumor mutation burden (TMB) [6]. However, many patients do not achieve an improved clinical outcome. Resistance to ICIs might be induced due to a variety of factors, most of which lead to inhibition of T-cell activity, either in a qualitative or a quantitative manner [7]. Thus, identifying comprehensive and reliable predictive biomarkers for ICIs in monotherapy or in combination with other therapeutics to improve clinical outcomes are urgently needed.

Multiple biomarkers have been reported to be associated with ICI response, including: TMB, mismatch repair (MMR)/microsatellite instability (MSI), PD-L1 expression measured using immunohistochemistry, circulating tumor DNA (ctDNA) and host factors (gender, age and HLA diversity) [8, 9]. A variety of biomarker strategies have been explored to further our understanding of mechanisms of immune response, however, tumoral complexity and heterogeneity limit the clinical utility of standard biomarker options.

In this issue of Annals of Oncology, Bareche *et al.* [7] report a pan-cancer meta-analysis using 3,648 patients with solid tumors treated with ICIs from 26 studies. As per previously published meta-analyses (**Table 1**), they evaluated a panel of biomarkers (TMB, gene signatures and genes) associated with response to immunotherapy, a topic with a high translational potential and broad interest to the medical community. These patients were divided into 2 large cohorts, a discovery and a validation cohort. Of all patients, 79.8% received anti-PD1 or anti-PD-L1 mAb, followed by anti-CTLA-4 mAb therapy (9.8% cases) and by a combination of the two (10.4% cases). As a result, they further confirmed that high TMB was associated with an improved ICI response, being associated with a better PFS (progression-free survival) and OS (overall survival). Nevertheless, in the case of renal cell carcinoma, it was observed that TMB was not significantly associated with response. Even though there was a significant heterogeneity, TMB was considered a robust biomarker for predicting ICI response.

Next the authors investigated gene expression signature(s) associated with sensitivity or resistance to ICI therapy. Bareche *et al.* [7] identified five clusters of signatures, of which three were related to chemokine, cytokine and T-cell receptor signaling pathways, the fourth related to antigen-presenting, and the fifth cluster related to cell cycle, pathways in cancer and extracellular matrix receptor. Consequently, 22 signatures were significantly associated with at least one of the clinical outcomes (response, PFS and OS) at pan-cancer level. Notably, ten ICI-sensitive signatures (i.e., STAT1, Inflammatory, IRG_Ayers, TIS, TLS, T_cell_inflamed, PDL1, IFNG, CD8_Jiang and CYT), as well as one ICI-resistance signature (PTEN_MITF) were significantly associated with all three clinical outcomes.

Taking advantage of the large well powered dataset, the authors developed PredictIO, as a new predictive signature of ICI-response, PredicitIO was found to outperform other expression-based signatures and TMB. PredictIO is based on the top 100 genes, ranked on their association with ICI response, with 77 associated with ICI sensitivity, and 23 associated with ICI resistance. Using gene ontology, the genes associated with sensitivity were associated with immune response regulation, whereas resistance genes were associated with epithelial-to-mesenchymal transition (EMT). PredictIO exhibited a strong and significant association with PFS and OS, and three individual genes (*RAB42, FJX1* and *SORCS2*) were found to be significantly associated with prognosis.

In addition, Bareche *et al.* [7] calculated the T-cell dysfunction score for each gene included in the PredictIO signature to identify potential novel therapeutic targets for overcoming ICI resistance. Based on T-cell dysfunction score, four PredictIO genes were identified, *F2RL1*, *GALNT5*, *RBFOX2* and *SOX9*, the first two associated with a worse response to ICIs in both discovery and validation cohorts, and also negatively associated with CD8 T cells and positively with M2-polarized macrophages. As a translational novelty of the work, the authors demonstrate the utility of "Big Data" in finding ICI resistance/sensitivity markers that can be exploited mechanistically, as biomarkers or therapeutically.

The performed meta-analysis follows a standard backbone on par with most PRISMA guidelines and using a larger cohort than previously used. Nonetheless, there were certain drawbacks to this, as the authors only used PubMed as a search engine and did not submit the proposal for the meta-analysis beforehand to PRISMA. The current meta-analysis included several cancer types treated with immunotherapy. Although this gave rise to a certain heterogeneity of the data generated, it also led to a more general application of the conclusions of the study and of the predictor generated by it. The PredictIO model presents a good performance by predicting gene expression signature in a pan-cancer study. As a further step, other cohorts should be assessed using PredictIO to determine its predictive capabilities.

In conclusion, given the tremendous importance and wide use of ICI, the generation of novel prediction biomarkers for response are utmost essential. Bareche *et al.* have not only performed a meta-analysis revealing the most important gene signatures involved in ICI sensitivity and resistance, but they have also generated a predictor for ICI response that outperformed TMB, a FDA approved biomarker for ICI response prediction. Further studies will highlight the significance of this important study.

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TABLE 1. Examples of meta-analyses on biomarkers for prediction of ICI response.

Source	# patients	# studies	Findings	References
Bareche Y et al. 2022	3648	26	Identified a novel gene expression signature – PredictIO used to predict ICI-response and identified potential biomarkers of interest. F2RL1 and RBFOX2 highly associated with ICI resistance, T-cell function and pro-tumoral TME.	[7]
Litchfield K et al. 2021	>1000	12	Strongest predictor for checkpoint inhibitors (CPI) are clonal TMB and CXCL9/CXCL13, as well as 9q34 loss and CCND1.	[10]
Wang Y et al, 2022	4289	22	PD-1 as the first-line treatment for non-squamous NSCLC patients shows promising results compared to chemotherapy.	[11]
Lu S, et al, 2019	8135	45	Multiplex immunohistochemistry/immunofluorescence (mIHC/IF) have significantly higher diagnostic accuracy than PD-L1, TMB, or gene expression signatures in predicting clinical response to anti–PD-1/PD-L1 therapy	[12]
Zhu K, et al, 2022	770	13	A high metabolic tumor volume is associated with a shorter survival in the case of NSCLC patients treated with ICIs.	[13]
Mao X, et al, 2022	5936	24	Peripheral IL8 levels could be predictive of ICI response.	[14]
Zhou K, et al, 2022	2312	21	In the case of advanced lung cancer patients, a low platelet to lymphocyte ratio was predictive of a better survival.	[15]