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RESEARCH HIGHLIGHT

Predictive immune biomarkers: an unattainable chimera?

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Immune checkpoint blockade (ICB) with antibodies interfering with cytotoxic T lymphocyte antigen 4 (CTLA-4) and the programmed cell death 1 (PD-1) protein tremendously revolutionized therapy for advanced cancer. Nevertheless, the activity of such agents (ipilimumab and nivolumab) is limited to a 10–45% response rate in the context of unselected populations with advanced solid tumors. Several common cancer types have demonstrated a very low frequency of response (breast, prostate and colon cancers), and heterogeneous responses have been observed between distinct tumors within the same patient. Challenges to broad clinical applicability include identifying patients most likely to benefit from checkpoint inhibitors and overcoming resistance to ICB. The immune response is a dynamic and constantly evolving process due primarily to patient-dependent factors, including genetic and tumor microenvironment (TME) features, and secondarily to treatment interventions. Thus, the anti-tumor immune response and the establishment of resistance mechanisms may pre-date immunotherapy challenge or may be induced by therapy.[1,2] In the past decade, the FDA approved ipilimumab, nivolumab, pembrolizumab, atezolizumab and durvalumab in rapid succession, unveiling the need for robust predictive biomarkers.[3,4] The TME affects the response to immunotherapy associated with 'inflamed' TME, and gene signatures associated with T cell-inflamed tumors have also predicted response. The association between TIL counts and response was demonstrated both before and after treatment with CTLA-4 or PD-1/PD-1 ligand (PD-L1) checkpoint blockade.[5,6] In tumors with constitutive PD-L1 expression, the predictive value of PD-L1 may be improved by adding additional parameters, such as infiltrating CD8+ T cells or an IFNγ gene signature.[7] However, these findings are not universal, and the dynamic and individual nature of the response makes these investigations difficult. PD-L1 measurement appeared to be the most logical marker for anti-PD-1-based therapy; the only approved companion diagnostic kit is for anti-PD-L1 in melanoma and non-small cell lung cancer. Nevertheless, PD-L1 expression cannot adequately summarize the complexity of the tumor–immune system interactions and consistently predict patient benefit from immunotherapy. In the interesting manuscript 'Predictors of responses to immune checkpoint blockade in advanced melanoma' in *Nature Communications*,[8] Jacquelot *et al.* utilized a previous cohort of 39 stage III/IV melanoma patients evaluated for blood and tumor tissue immune parameters. In detail, they analyzed 25 paired tumor/blood samples and evaluated 124 parameters in blood and 128 in tumors for a total 252 parameters, demonstrating that blood markers were as contributive as tumor-infiltrated lymphocyte immunotypes. In addition, parameters associated with lymphocyte exhaustion/suppression exhibited increased clinical significance compared with those related to activation or lineage.[9] In the current manuscript, the parameters derived by the first study are integrated with data obtained from lymph nodes from 37 stage III melanoma patients exposed to the treatment reported in the panel for a total of 779 blood/tumor parameters. The treatments mainly targeted immune checkpoint receptors, such as CTLA-4, PD-1 and TIM-3 plus control treatment conditions (IL-2, IFNα 2A or mIgG1) (Figure 1). A sophisticated biostatistics evaluation defined biological parameters associated with responders vs non-responders to the tested settings. Moreover, the obtained parameters were retrospectively validated in a couple of external cohorts of patients treated with ipilimumab or ipilimumab plus nivolumab using blood samples obtained at the diagnosis. With its complexity,

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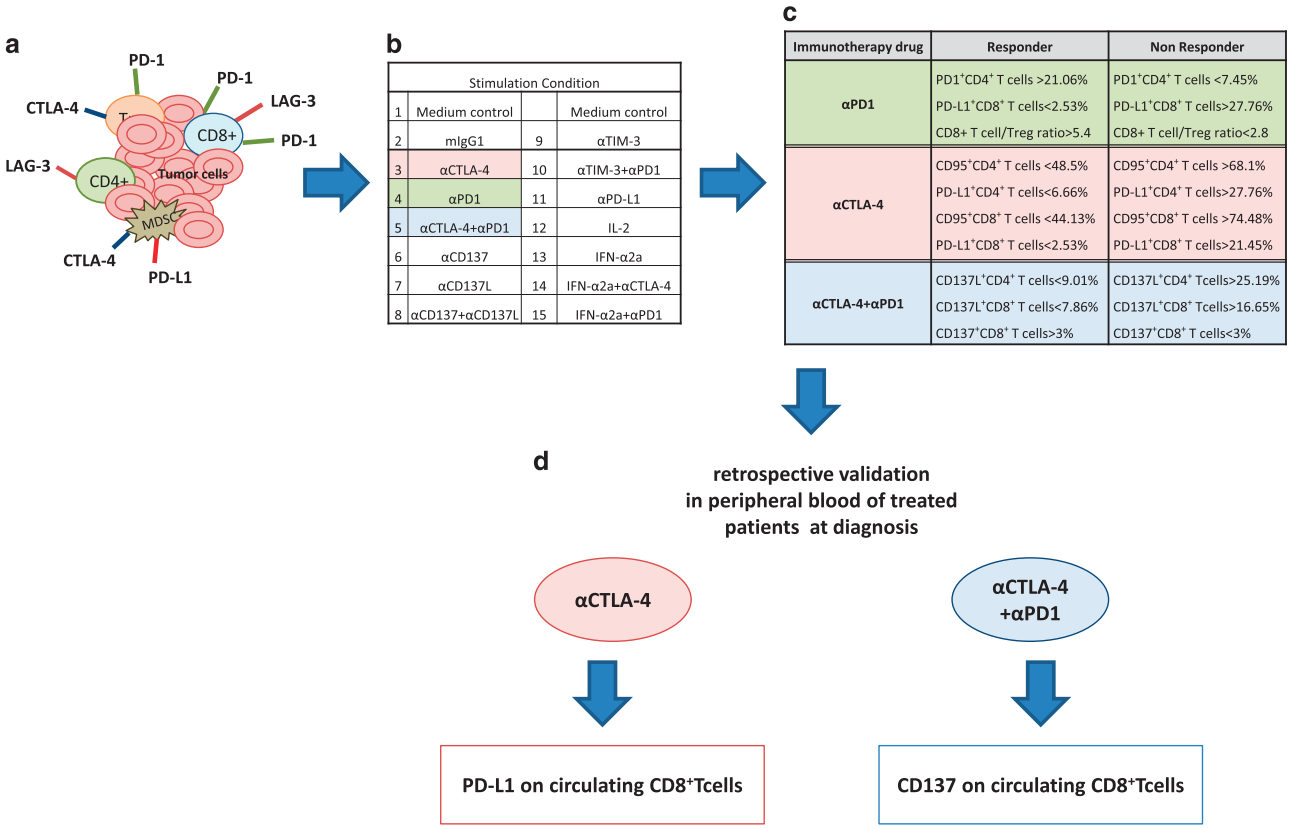

Figure 1 Immune biomarker identification: key points in the process. (**a**) Culture cells from lymph nodes derived from stage III melanoma patients. Schematic of tumor and tumor microenvironment. (**b**) Table of the treatments to which the cells in **a** were exposed. (**c**) Identification of parameters that discriminate patients as 'Responder' vs 'Non-responders' to anti-PD-1, anti-CTLA-4 and both among the conditions evaluated in **b**. (**d**) Retrospective validation of the parameters identified in **c** in the blood of resected stage IIIc and IV melanoma patients from a phase 2 adjuvant trial of ipilimumab plus nivolumab.

this manuscript exhaustively represents the task to identify suitable biomarkers of immunotherapy. Through sequential steps of validation, the authors elegantly discovered settings that most likely reproduce patient heterogeneity, with even more complexity with CTLA-4 or CTLA-4 plus PD-1 targeting. Obviously, the ideal biomarker is a blood biomarker. Importantly, the authors confirm that the peripheral markers are as reliable or even more reliable than tumor-derived markers. The peripheral evaluation of PD-L1 and CD137 (4-IBB) on circulating CD8 is an easy and accessible task to be validated in perspective longitudinal studies of ipilimumab and nivolumab patients and retrospectively from ongoing studies. This manuscript represents a clear example of the centrality of system biology in the age in which big data need to be analyzed and interpreted. Owing to technological developments, it is possible to simultaneously evaluate multiple cellular features that can enhance the resolution of the examined biological condition. The combined tasks of flow cytometry and mass spectrometry generated the mass cytometry that provides measurements of greater than 40 simultaneous cellular parameters at the single-cell resolution, significantly augmenting the ability of cytometry to evaluate complex cellular systems and processes. In a widely used spontaneous model of murine carcinoma, MMTV-PyMT (murine mammary tumor virus-polyoma middle T) triple-negative breast cancer refractory to other immunotherapies, such as anti-PD-1, the effect of intratumoral immunotherapy was monitored in several tissues through mass cytometry. Systemic and tumoral immune responses were present shortly after administration of effective therapy. However, during tumor rejection, only systemic immune cell proliferation was evident. A specific subset of peripheral CD4 T cells were increased in patients responding to immunotherapy and provided protection against new tumors.[10] To shed further insight into the underlying mechanisms of anti-CTLA-4- and anti-PD-1-induced tumor rejection, mass cytometry was used to comprehensively profile the effects of checkpoint blockade on tumor immune infiltrates in human melanoma and murine tumor models. A restricted number of differences were revealed between tumor-infiltrating T cell populations between tumor models, which indicated that checkpoint blockade targets only specific subsets of tumor-infiltrating T cell populations. Anti-PD-1 predominantly induces the expansion of specific tumor-infiltrating exhausted-like CD8 T cell subsets, whereas anti-CTLA-4 induces the expansion of an ICOS+ Th1-like CD4 effector

population in addition to engaging specific subsets of exhausted-like CD8 T cells.[11]

How informative are these phenotypes in monitoring and predicting an immunotherapy efficacy? Only a coordinated, perspective clinical trial will provide the answer. Moreover, do we really need to scan and fish in these complex seas represented by each individual immune system in an attempt to extrapolate some common biomarkers of response? On 23 May 2017, the FDA approved the PD-1 inhibitor pembrolizumab for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch-repair-deficient (dMMR) solid tumors regardless of tumor site or histology. This approval was based on common characteristics, including lymphocytic infiltration, somatic hypermutation and increased neoantigen formation shared by MSI-H and dMMR. Of note, the incidence of MSI-H or dMMR is ~30% for endometrial cancer, 20% for colon or gastric cancer, and <5% for most other tumor types, with even lower numbers for MSI-H or dMMR status in secondary lesions.[12]

In contrast, in 2017, the FDA approved two PARP inhibitors, olaparib and niraparib, as maintenance treatments for women with ovarian cancers who respond to induction platinum-based chemotherapy regardless of their BRCA-mutation status. The phase III ARIEL3 trial of rucaparib confirmed the genotype agnostic benefit of PARP inhibition with improved disease progression across subgroups in which a benefit was expected (women with somatic BRCA alterations or BRCA wild-type, LOH-high disease) vs groups in which a benefit was not expected (patients with LOH-low tumors).[13] Although it is evident that toxicity and costs represent crucial issues, the definition of biomarkers in this field is still difficult, and 'listening to the shell sea' derived from treated patients' biopsies represents the only method to include all the notable parameters. In conclusion, the work from Jacquelot *et al.* helps in designing an elegant and exhaustive, although complex, algorithm toward the identification of powerful, predictive biomarkers to optimize ICB therapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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