Editorial

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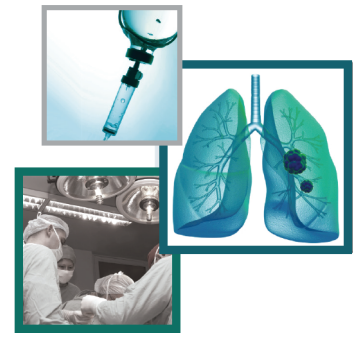
Lung Cancer Management



Systemic immunological biomarkers of clinical responses in immune checkpoint blockade therapies

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"The discovery of immune checkpoint interactions as a strategy used by cancer cells to inhibit the antitumor capacities of the immune system, and the development of drugs blocking these interactions, have revolutionized the treatment of lung cancer"

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The discovery of immune checkpoint interactions as a strategy used by cancer cells to inhibit the antitumor capacities of the immune system, and the development of drugs blocking these interactions, have revolutionized the treatment of lung cancer. In NSCLC, immunotherapy targeting PD-1/PD-L1 interactions has become standard for platinum-refractory patients and for first-line treatment of metastatic tumors. Immune checkpoint blockade is applied as single therapy, in combination with chemotherapy or as consolidation therapy after chemoradiotherapy in locally advanced tumors (KEYNOTE-189) [1]. Some recent data from clinical trials (NCT02763579) [2] demonstrate that immune checkpoint blockade might improve survival of patients with small-cell lung cancer.

Immune checkpoint blockade does extend survival compared with chemotherapy regimens [1,3]. However, most of the benefit is hoarded by a small proportion of patients, exhibiting long-term disease control which does not usually go above 20%. Importantly, hyperprogressive disease has been described in a proportion of patients for whom there is an acceleration of tumor growth following immunotherapies [4]. This seems to be especially true for lung cancer. For these reasons, the finding of predictive biomarkers of responses to immune checkpoint blockade constitutes a major research issue of critical importance in oncology. Due to this necessity, extensive research is currently being carried out to identify biomarkers of responses. Most studies utilize 'open' nonbiased approaches for the identification of these biomarkers. This has been strongly promoted by the development of high-throughput analytic techniques such as CyTOF, liquid biopsies and next-generation sequencing (NGS). These techniques allow the simultaneous quantification of multiple parameters and cell lineages. Even the use of machine learning is being applied to identify the relevant biomarkers from vast amounts of data that are not apparent to the day-to-day biomedical researcher and oncologist.

One of the first biomarkers to be tested was PD-L1 tumor expression, as this molecule is a direct target of anti-PD-L1/PD-1 blockade therapies. Indeed, there is a correlation between the percentage of PD-L1-expressing cells and clinical benefit in NSCLC. However, the predictive value of PD-L1 expression varies between drugs and tumor histology. This was apparent in CheckMate 017 and CheckMate 057 for the treatment of squamous and nonsquamous NSCLC [5]. Such variety was even shown with the same drug in similar patient cohorts such as in Phase II POPLAR (NCT01903993) [6] and Phase III OAK (NCT02008227) [7] with atezolizumab. Moreover, no association has been demonstrated between PD-L1 expression and efficacy in small cell lung cancer

Future Medicine

(NCT02763579) [2]. The interpretation is even more challenging when PD-1/PD-L1 checkpoint blockade is combined with chemotherapy or antiangiogenic drugs, as many factors might influence the outcomes. Inconsistency between trials could be explained by the fact that PD-L1 is a dynamic marker, characterized in many instances by heterogeneous expression within the tumors and even in immune infiltrates. In addition, the time elapsed between the biopsy and the start of immunotherapies can cover months and may not represent the current status of the tumors in the patient.

Tumor mutational burden (TMB) is associated with better clinical outcomes [8], although so far the data is still scarce. In fact, TBM was a better predictor than PD-L1 tumor expression for selecting patients undergoing iplimumab/nivolumab combination in Phase III CheckMate 227. However, TMB evaluation is very costly compared to PD-L1 immunohistochemistry when performed through whole-exome sequencing (WES). NGS algorithms have been developed for clinical use, and even a NGS-based *in vitro* diagnostic test under the name FoundationOne CDx™ has been approved by the US FDA. Nevertheless, the elevated cost of these techniques appears to be a significant limitation for its routine application.

In the last decade, immune checkpoint blockade-based immunotherapy has caused a change of paradigm in oncology. Now the target of the treatments is fundamentally the immune system and not solely the tumor. Hence, the components and regulatory mechanisms of the immune system will deeply condition the efficacy. This has been previously shown in numerous preclinical immunological studies before immune checkpoint blockade was applied in human therapy. It is reasonable to revise all previous extensive work on cancer immunotherapy for cherished predictive biomarkers or clues of what these biomarkers might be. Some efforts have been put toward this goal. The quantification of tumor infiltrating lymphocytes as a prognostic biomarker has been known for decades. Indeed, more technically developed methods based on tumor infiltrating lymphocyte (and other immune cell infiltrates) quantification have been developed for predicting clinical outputs in immune checkpoint blockade therapies. The immunoscore is probably one of the most widely used methods [8,9]. However, the technical difficulties in attaining a precise and reproducible evaluation strongly limits its application. Undoubtedly, the use of systemic biomarkers would ease up their routine clinical use, particularly those quantified from small peripheral blood samples. The neutrophil-to-lymphocyte ratio has also been known for several years to be a prognostic biomarker for many cancer types. A meta-analysis study revealed the utility of neutrophil-to-lymphocyte ratio as a prognostic biomarker in NSCLC, but not so much for small-cell lung cancer [10]. The development of high-throughput analytical techniques allows for the more precise quantification of immune cell populations. Multicolor flow cytometry is inexpensive, and can be translated easily to routine clinical practice. An elegant translational work by Kamphorst *et al.* showed that CD8 CD28 T-lymphocyte expansion occurred in patients who responded to anti-PD-1 therapy [11]. This is a demonstration of a dynamic direct activity of anti-PD-1 antibodies over systemic T-cell populations. Another high-throughput study from peripheral blood using CyTOF and machine learning showed that responder patients had increased baseline percentages of $CD14^+$ monocytes [12]. However, the differences between nonresponder and responder patients were minimal and possibly cannot be detected by standard approaches. In addition, the very high cost of CyTOF makes it difficult for routine clinical use at the moment.

Our group has been interested in the study of fundamental immunological anticancer mechanisms for several years, particularly the role of PD-L1/PD-1 interactions on cancer cells and T-cell responses [13,14]. The role of CD4 T-cell responses in antitumor immunity has been controversial over the decades but most of the preclinical evidence highlights their key contribution. Let us not forget that CD4 T cells are main regulators of CD8 T cells during antigen presentation. Recent data is confirming the importance of neoantigen-specific CD4 T cells for efficacious antitumor immunity [15]. In a recent work, we have monitored the dynamics of different CD4 T-cell populations systemically in peripheral blood of NSCLC patients who are undergoing anti-PD-L1/PD-1 therapies as second and third-line treatment. Interestingly, we found that patients could be divided in two groups according to the baseline percentage of highly differentiated memory CD4 T cells [16]. Patients with low percentages of this CD4 T-cell subset before the start of therapy did not show objective responses to PD-1/PD-L1 immunotherapy. Indeed, the overall response rate was of 0%. In contrast, patients with high baseline percentages of memory CD4 T cells showed overall response rates of 50%, and close to 70%–80% when combined with PD-L1 positivity. Hence, CD4 T-cells may be playing a more direct role in the efficacy of anti-PD-L1/PD-1 immunotherapies than previously anticipated.

Indeed, dynamic changes of CD4 T-cell populations could be successfully used for 'real time' monitoring of responses from small blood samples during immunotherapies. Information on dynamic changes in systemic immune populations could complement radiological evaluation in lung cancer patients and help making informed

decisions [11,12,16]. Quantification of systemic immunological parameters could be used to stratify patients before the start of immunotherapy [16], not only of immune populations but also cytokines such as IL-8 [17]. Moreover, information from immunological variables will shed light on the faulty immunological mechanisms present in nonresponders. This can open the door to therapeutic approaches that can successfully complement immune checkpoint blockade and increase response rates for lung cancer.

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