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Commentary

Efficacy of PD-1 checkpoint inhibitor therapy in melanoma and beyond: are peripheral T cell phenotypes the key?

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Summary

Immunotherapy treatment strategies have proven effective in a limited portion of patients, where identifying responders from non-responders to treatment remains a challenge. While some indications can be drawn from invasive biopsies, we need more accessible methods for predicting response and better correlates of response prior to starting therapy. Recent work has identified differences in immune composition at baseline in peripheral blood from melanoma patients responding to PD-1 blockade treatment. Through flow cytometric analysis of T cell receptors, phenotypical features of CD8+ and CD4+ T cells and Tregs could allow for the stratification of treatment response. Analysing T cells within peripheral blood could potentially allow for the stratification of PD-1 treatment response prior to therapy in different cancer settings.

Keywords: PD-1, checkpoint, inhibition, cancer

Introduction

A successful immune response lies in the balance between effective immunity and avoiding unwanted immunopathology. Two co-inhibitory receptors found on T cells are PD-1 and CTLA-4, which are up-regulated upon T cell activation and are involved in dampening the T cell response to tightly control the immune system [1]. During chronic inflammation, in particular, T cells are known to become exhausted and overexpress these receptors [1]. In the context of cancer, one of the many mechanisms deployed by the tumour to evade the immune response is the upregulation of co-inhibitory ligands, such as PD-L1 with the aim of downregulating T cell activity [2], where targeting these receptors established a novel way of treating cancer and changed the field of oncology. CTLA-4 and PD-(L)1 checkpoint inhibitors are used to treat numerous cancers and have been shown to improve response rates, progression-free and overall survival compared with cytotoxic chemotherapy [3]. Despite this success, many patients become resistant after the initial response or do not respond at all [4], and checkpoint blockade comes with the risk of autoimmune adverse events [5], highlighting a need to identify patients likely to respond to immunotherapy or biomarkers of success/resistance [6].

Due to the heterogeneity of response to checkpoint blockade, a few practices are implemented to predict patient response. Multiple studies have shown that baseline expression of PD-L1 by immunohistochemistry is predictive of response to PD-1 checkpoint blockade [4]; however, patients with PD-L1 negative tumours may also respond to therapy, making PD-L1 not a widely used biomarker for patient stratification [7]. High tumour mutational burden [8] and the presence of tumour-infiltrating lymphocytes have also been shown to associate with response to checkpoint blockade [9]. All of these biomarkers require tumour biopsies or fine needle aspirates. Additionally, due to tumour heterogeneity, a single biopsy may not provide enough information to enable an accurate understanding of the tumour phenotype, emphasizing the need for an improved and minimally invasive method for predicting response [10]. Numerous studies have found tumour-specific T cells in the circulation as well as proliferating T cell clones following PD-1 blockade treatment [11]; one such study found circulating Ki-67+ PD-1+ CD8+ T cells to correlate with response to PD-1 blockade [12]. Although CD8+ T cells directly target tumour cells, CD4+ T cells are also involved in tumour cytotoxicity, senescence, and the destruction of the tumour vasculature [12]. The few studies that have explored the presence of biomarkers in *ex vivo* peripheral blood lymphocytes have focused primarily on CD8+ T cells, highlighting the need to investigate the phenotype of both CD4+ and CD8+ T cells [13, 14].

Peripheral blood immune phenotyping

In a recent issue of Immunotherapy Advances, Edner *et al.*, immunophenotyped peripheral blood samples from 20 patients with advanced malignant melanoma before and

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after treatment with Pembrolizumab, a PD-1 blocking antibody [15]. The study investigated correlations of response and progression after PD-1-directed therapy with T cell phenotype by flow cytometry analysis. Edner *et al*., not only found post-treatment immune correlates of PD-1 blockade efficacy but crucially describe differences in immune composition between responders and non-responders prior to receiving therapy.

Non-responders showed incomplete PD-1 blockade on CD4+ T cells, where individuals who failed to respond to PD-1 blockade had an increased frequency of peripheral blood PD-1+ CD4+, but not PD-1+ CD8+ T cells, at 6 weeks post therapy. Additionally, PD-1 blockade induced distinctive immune changes between responders and non-responders. Responders had an increased frequency of proliferating CD28+Ki67+CD8+ T cells at 6 weeks, whilst non-responders had increased frequencies of activated and proliferating regulatory T cells (Treg).

Clinical response to PD-1 blockade can be distinguished prior to therapy

Responders showed higher baseline CD4+ and CD8+ T cell proliferation and T cell compartments predominated by naive and central memory cells whilst non-response was associated with effector memory and terminally differentiated subsets. Non-responders also showed an increased baseline expression of the inhibitory receptors 2B4 and KLRG1. Although in responders, only the CD8+ T cells showed a significant increase in proliferation marker Ki67 following treatment with Pembrolizumab, CD4+ T cells also showed a trend toward increased Ki67 expression, suggesting that PD-1 blockade has a greater impact on CD8+ than CD4+ T cells. This finding is supported by previous evidence from Kamphorst *et al*. in the context of lung cancer, where PD-1 and CTLA4 blockade had a greater impact on CD8+ than CD4+ T cells [14]. Responders at baseline may have more antigen-experienced stem-cell-like CD8+ T cells compared to exhausted CD8+ T cells, similar to previous reports from the Ahmed group [16]. Stem-cell-like CD8+ T cell phenotypes predominantly respond to PD-1 blockade, whereas non-responder CD8+ T cells upregulate co-inhibitory receptors, such as 2B4 and KLRG1, which are associated with terminally exhausted CD8+ T cells. Collectively, these data suggest that responders may have a skewed stem-cell-like PD-1+ CD8+ T cell phenotype that responds to PD-1 blockade, whereas PD-1+ CD8+ T cells in non-responders have a more exhausted phenotype.

Conclusion

The immunophenotyping of peripheral blood in patients with advanced malignant melanoma treated with pembrolizumab in this study, both at baseline and following treatment, has revealed immune biomarkers that correlate with response to treatment. Unlike previously published studies, Edner *et al*. analysed both CD4+ and CD8+ T cells and found the phenotype of CD4+ T cells following treatment to also play a role in indicating response, highlighting the importance of analysis of both T cell subtypes within peripheral blood. Additionally, this study identified immune phenotypes in the peripheral blood of patients prior to receiving PD-1 blockade, which were indicative of response, such as higher baseline CD4+ and CD8+ cell proliferation in responders compared with non-responders. Even though this study included a small cohort of patients and further validation in a larger cohort is important, the findings are well supported by previously published work. Overall, the work from Edner *et al*. has demonstrated the power of analysing specific peripheral T cell phenotypes that could be predictive of treatment response. Peripheral blood samples could provide an easily accessible method for reliable biomarker detection and are worth further investigation in the setting of immunotherapy response.

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Author contributions

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Conflict of interest

The authors have no conflicts of interest.

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