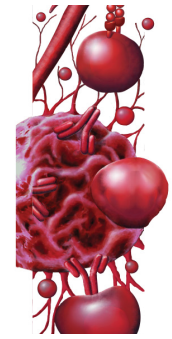
Editorial

Immunotherapy



BCL-2-interacting mediator of cell death (Bim) is a novel biomarker for response to anti-PD-1 therapy in patients with advanced melanoma

"Measurement of Bim levels in tumor-reactive PD-1$^+$ CD8 T cells may represent a novel and less invasive strategy to predict and monitor responses to anti-PD-1 blockade therapy..."

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Despite major developments in medical technologies, durable responses in patients with advanced cancers, including malignant melanoma, remain frustratingly low. While many agents with divergent biological mechanisms of action are capable of causing complete responses, they do so in a seemingly random fashion and only in a subgroup of patients. It is still unclear what ultimately predicts 'responders' from 'nonresponders', although the existence of a common 'biologically limiting step' dependent not only on the mutational profile of cancer cells, but also on the permissive/supportive actions of the host immune system is increasingly postulated. Recently, the pathway of programmed cell death 1 (PD-1) and its ligand PD-L1 (aka B7-H1) has been found to play an important role in tumor-induced immunosuppression in melanoma and other malignancies, and is an increasingly exploited therapeutic target [1–4]. The key challenges in targeting PD-1 or PD-L1 therapy are the low response rate and the lack of reliable biomarkers to predict or monitor therapeutic responses.

Anti-PD-1 or PD-L1 blockade aims to restore antitumor immunity by impeding interactions of the PD-1 receptor expressed by tumor-reactive T cells with PD-1 ligands (e.g., PD-L1/B7-H1) expressed by tumor cells [3]. Clinical trials with PD-1 and PD-L1 blockade have demonstrated promising therapeutic responses in 18–40% of patients with advanced malignancies, including melanoma [1,2,5]. Two anti-PD-1 monoclonal antibodies have been approved by the US FDA for the treatment of patients with metastatic melanoma and metastatic non-small-cell lung cancer. Pembrolizumab was also recently approved for the treatment of patients with recurrent or metastatic head and neck squamous cell carcinoma, while nivolumab is approved to treat patients with advanced (metastatic) renal cell carcinoma. In addition, an anti-PD-L1 monoclonal antibody (atezolizumab) has recently been approved for the treatment of patients with locally advanced or metastatic urothelial carcinoma whose disease has worsened during or following platinum-containing chemotherapy [6]. However, clinical outcomes are variable in that some patients achieve rapid and durable complete responses to primary anti-PD-1/anti-PD-L1 therapy or to its re-induction, other patients experience progression followed by significant reduction in tumor burden with continued therapy and some patients show no clinical benefit. Indeed, these immune checkpoint-blocking agents typify the phenomenon of dramatic therapeutic responses in a subset of patients who cannot be pre-identified, necessitating broad treatment application in an unselected patient population. In addition,

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some patients experience pseudoprogression with an ostensible enlargement of tumors related to immune cell infiltration that precedes a therapeutic response and an ultimately favorable clinical outcome [7]. Given the unconventional response patterns seen with immunotherapeutic agents, alternative methods of evaluating tumor response and progression are warranted along with the immune-related response criteria [8]. As of now, there is no validated biomarker for clinicians to identify patients who may ultimately benefit from immunotherapies.

Although the presence of new tumor antigens (i.e., neoantigens) [9] and increased infiltration of tumor tissues with lymphocytes [10] are predictive for the pre-existing immune responses to tumors favoring immunotherapy in general, there is no prospective biomarker to reflect how PD-1$^+$ tumor-reactive T cells respond to anti-PD-1 blockade in particular. We argue that the sensitivity of PD-1$^+$ T cells to anti-PD-1 therapy is influenced by the status of PD-1 engagement with its ligands. Measurements of this engagement determine the reversibility of PD-1$^+$ T cells from dysfunctional to functional T cells. As currently conceptualized, when activated PD-1$^+$ T cells encounter PD-1 ligands (i.e., B7-H1/PD-L1) on tumor cells, intracellular signaling events are initiated resulting in either T-cell death [11] or an 'exhausted' nonfunctional state [12]. The question remains how to distinguish PD-1 expressing T cells that have engaged PD-L1 and have become functionally compromised, from those that have yet to fully engage their ligand and could theoretically be reversed.

We recently described Bim (BCL-2-interacting mediator of cell death) as a marker of the status of T-cell PD-1 engagement by its ligands, which identifies patients who are more likely to benefit from anti-PD-1 therapy [13]. We found that Bim levels in PD-1$^+$ immune T cells geared toward fighting cancer reflect their engagement with a key immune molecule (PD-L1) used by tumors to escape the body's immune attack. Bim levels in tumor-reactive T cells of patients with metastatic melanoma being treated with anti-PD-1 therapies such as pembrolizumab may *a priori* identify patients who would derive clinical benefit from this treatment, and provide a noninvasive way to monitor responses to treatment. We reported that patients who experienced tumor shrinkage or stabilization after four cycles of anti-PD1 therapy had higher levels of Bim in circulating, tumor-reactive (PD-1$^+$ CD11a^{high} CD8$^+$) T cells at baseline compared with patients who experienced tumor growth, likely reflecting an abundant interaction of PD-1 on immune cells with its tumor-associated ligand PD-L1 (B7-H1) [13]. Our findings are in agreement with previous reports of higher objective responses to anti-PD-1 therapy in patients with PD-L1-positive tumors [14], with higher expression levels capturing the most PD-1-responsive population [10]. In addition, we found that Bim levels decreased significantly after the first 3 months of treatment in responders compared with nonresponders, indicating tumor regression and therefore less PD-1 engagement with tumor-associated PD-L1. Therefore, increased levels of Bim in T cells before treatment may predict that there is more ongoing PD-1/PD-L1 interaction and that these patients are more likely respond to therapy. During treatment, decreased levels of Bim suggest that immune checkpoint inhibitors may have successfully blocked the interaction between PD-1 and PD-L1 in tumor-reactive T-cell populations and may even precede objective assessment of clinical outcomes (such as tumor size reduction on imaging tests or prolonged survival).

On the other hand, the lower frequency of Bim$^+$ CD8$^+$ T cells in nonresponders [13] suggests either the availability of fewer PD-L1 engaged effector T cells or decreased tumor antigen stimulation; in either case, PD-1 blockade was not be able to make a significant impact because of limited numbers of tumor-reactive T cells in this clinical context. Interestingly, after 12 weeks of treatment, the frequency of Bim$^+$ CD8$^+$ T cells either did not change significantly or increased in nonresponders, suggesting that anti-PD-1 antibody could not efficiently block the interaction of PD-1/PD-L1 or other immune regulatory mechanisms may be at play. Importantly, our results suggest that biological alterations in tumor-reactive T cells isolated from the peripheral blood may indicate what happens in tumor tissues. Future studies are warranted to validate the correlation between the functional changes in circulating T cells and in tumor-infiltrating T cells.

Clinical outcomes with the novel immunotherapeutic agents such as anti-PD-1 antibodies can be heterogeneous and unpredictable, such as delayed responses and prolonged disease control after an initial 'pseudoprogression'. Analysis of the time to response to pembrolizumab in clinical trials indicates that although most responses occur by week 12, some responses may also occur late in the course of treatment and were observed as late as 36 weeks. In addition, 8–10% of patients experienced pseudoprogression with ≥25% increase in tumor burden that was not confirmed as progressive disease on subsequent imaging, and these patients had favorable clinical outcomes. The challenge for clinicians is to separate true progression from pseudoprogression and therefore identify those patients who may ultimately benefit from PD-1 blockade despite initial apparent disease worsening. Among two patients with pseudoprogression in our study, we

measured a dramatic decline in the levels of Bim expression in CD8$^+$ T cells at 12 weeks while scans suggested that there was progressive disease in the metastatic sites. This decline in Bim levels was further validated at 16 weeks before imaging eventually confirmed improvement in these lesions at 36 weeks. Although it is not clear how the decrease of Bim$^+$ T cells is correlated with an enlarging tumor lesion, our observations suggest that PD-1 blockade may not only disrupt the PD-1 signaling but may also increase the retention or expansion of T cells in tumor sites. The increased T-cell infiltration eventually leads to tumor rejection as shown in follow-up imaging. To that end, measurement of Bim levels may be a useful marker to monitor objective responses to anti-PD-1 therapy, especially in patients who might have radiographic pseudoprogression of disease.

Since changes in biological processes may precede clinical signs of response, the discovery of biomarkers that predict sensitivity to treatment such as Bim is vital by not only informing clinical decision-making, but also helping to select patients with melanoma (and possibly other malignancies) who are most likely to benefit from PD-1 blockade. Measurement of Bim levels in tumor-reactive PD-1$^+$ CD8 T cells may represent a novel and less invasive strategy to predict and monitor responses to anti-PD-1 blockade therapy, although these findings need to be validated in larger groups of patients with melanoma and other malignancies (lung, kidney, bladder, etc.).

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