B cells as biomarkers: predicting immune checkpoint therapy adverse events

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Immune checkpoint inhibitors are becoming a cornerstone of cancer immunotherapy as a result of their clinical success in relieving immune suppression and driving durable antitumor T cell responses in certain subsets of patients. Unfortunately, checkpoint inhibition is also associated with treatment-related toxicities that result in a myriad of side effects, ranging from mild and manageable to severe and debilitating. In this issue of the *JCI*, Das and colleagues report an association between early therapy-induced changes in circulating B cells and an increased risk of high-grade immune-related adverse events (IRAEs) in patients treated with checkpoint inhibitors that target cytotoxic T lymphocyte–associated antigen-4 (CTLA4) and programmed cell death protein 1 (PD1). These findings identify potential predictive biomarkers for high-grade IRAEs that may be leveraged to improve patient monitoring and may prompt new treatment strategies to prevent IRAEs.

Checkpoint blockade therapy: the good, the bad, and the toxic

FDA approval of the use of immune checkpoint inhibitors for melanoma, head and neck cancer, non–small-cell lung cancer, urothelial carcinoma, and renal cell carcinoma has transformed clinical oncology within the past decade, and this class of therapies continues to undergo extensive evaluation for the treatment of a broad spectrum of additional tumor types. The principal goal of checkpoint inhibition is to bolster $CD8^+$ T cell cytotoxic effector function by relieving inhibitory brakes that, while critical for maintaining self-tolerance, prevent optimal T cell activation in response to malignancy (1). The substantial promise of checkpoint inhibition is reflected in the improved survival outcomes observed in the CheckMate 067 clinical trial (ClinicalTrials.gov NCT01844505) that evaluated combination treatment with ipilimumab, an anti-cytotoxic T lymphocyte–associated antigen-4–targeted (CTLA4-targeted) mAb, and nivolumab, a mAb targeting programmed cell death protein 1 (PD1), in patients with previously untreated advanced melanoma (2). Patients who received combination therapy or nivolumab monotherapy had three-year overall survival rates of 58% and 52%, respectively, with 19% of patients in the combination arm showing complete responses (2).

Despite the undeniable clinical success of anti-CTLA4 and anti-PD1 mAb therapy thus far, especially in highly immunogenic cancers such as melanoma, several challenges remain. A current paucity of biomarkers limits the ability to predict who will respond to these immune therapies (3) and, importantly, who will develop treatment-related autoimmune toxicity, which is a serious concern for the majority of treated patients. Such toxicities, known as immune-related adverse events (IRAEs), vary in severity and in the organ systems affected. Patients receiving checkpoint inhibitors have a significantly higher risk of developing IRAEs than do patients receiving other forms of therapy, and combined anti-CTLA4 and anti-PD1 mAb therapy leads to a higher incidence of all-grade and high-grade (grade ≥3) IRAEs than does either agent alone (2, 4–6).

IRAEs associated with anti-CTLA4 and anti-PD1 mAb therapy most commonly impact the skin, gastrointestinal, and endocrine systems and manifest as a variety of conditions, such as rash, pruritus, vitiligo, diarrhea, colitis, and thyroid dysregulation (7). Although IRAE symptoms are usually manageable and reversible, they frequently result in either treatment interruption or dose reduction and/or discontinuation of checkpoint therapy. As an example of the prevalence and challenge of IRAEs, 96% of patients in the ipilimumab and nivolumab combination arm of the CheckMate 067 trial experienced at least one IRAE (any grade), with 30% of these patients discontinuing treatment as a direct consequence of their IRAEs (2). Although it has been reported that neither IRAE management with immune-suppressive corticosteroids nor discontinuation of therapy because of IRAEs markedly interferes with a durable clinical response to anti-CTLA4 and anti-PD1 mAbs (2, 8), strategies to reduce patient morbidity stemming from IRAEs are desirable. Unfortunately, the specific immune mechanism(s) that drive IRAEs are unclear, and clinical strategies to predict and prevent high-grade IRAEs are lacking.

Circulating B cell abundance correlates with IRAE risk

T and B lymphocytes are critical mediators of autoimmunity and are thus implicated in IRAE pathogenesis. Recent studies have revealed that changes in circulating T cell repertoires in ipilimumab-treated patients

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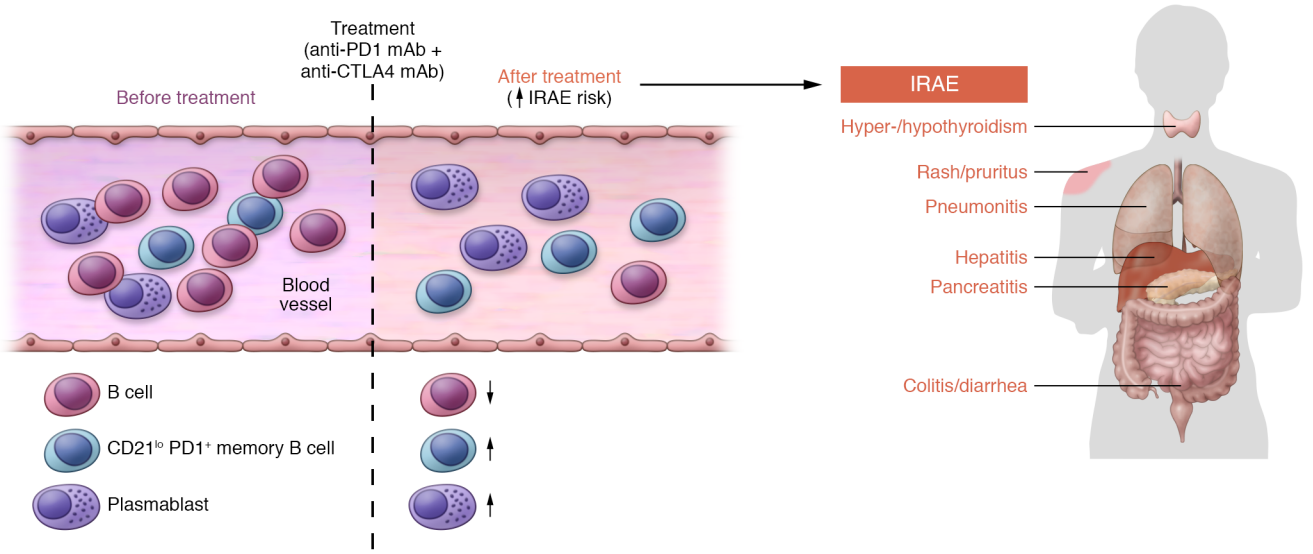

Figure 1. Changes in circulating B cells predict IRAE risk in patients receiving combined anti-CTLA4 and anti-PD1 therapy. Patients showing changes in circulating B cells after one cycle of combination anti-CTLA4 and anti-PD1 therapy (compared with their pretreatment baseline) have an increased risk of developing high-grade IRAEs. Specifically, a post-treatment reduction in total peripheral B cells and a coincident enrichment of differentiated $CD21^{lo}$ $PD1^{+}$ memory B cells and plasmablasts correlate with subsequent IRAE development. B cell changes are a unique immune biomarker of IRAE risk, as early changes in the frequency of other circulating leukocyte populations were not detected after therapy (data not shown). Select high-grade IRAE pathologies associated with combined anti-CTLA4 and anti-PD1 mAb therapy are depicted, and patients who showed B cell changes had a median three-week time to onset of one or more such IRAEs.

preceded the development of IRAEs (9, 10). While changes in T cell genomic signatures in patients undergoing anti-CTLA4 and anti-PD1 mAb treatment have also been identified (11), changes in B cells during checkpoint inhibition have not been previously reported.

In this issue, Das et al. analyzed circulating B cells in a small cohort of patients with advanced melanoma before and after treatment with anti-CTLA4 and anti-PD1 mAbs, administered as single agents or in combination (12). They found that a reduction in total peripheral B cells after a single cycle of combined checkpoint blockade (CCB) coincided with enrichment of plasmablasts and a proliferative $CD21^{lo}$ $PD1^{+}$ memory B cell subset. Single-cell RNA sequencing of $CD21^{lo}$ $PD1^{+}$ B cells collected from a patient prior to and after CCB revealed increased transcription of genes associated with cell activation and inflammatory cytokine production following treatment. $CD21^{lo}$ B cells also expressed lower levels of the lymphoid tissue–homing chemokine receptors CXCR4 and CXCR5 as compared with $CD21^{hi}$ B cells, indicating that $CD21^{lo}$ cells may have a greater capacity to traffic to nonlymphoid tissues and contribute to inflammatory processes that may mediate autoimmunity.

Given these findings, Das et al. developed a metric to evaluate whether changes in the frequency of circulating B cells in CCB-treated patients correlated with an increased risk or severity of IRAEs. Using this metric, the authors found that patients with a 30% or greater reduction in baseline levels of total circulating B cells and a two-fold or greater increase in $CD21^{lo}$ B cells or plasmablasts were significantly more likely to develop high-grade IRAEs than were patients without B cell changes (Figure 1). Moreover, early changes in circulating B cells after only one round of CCB correlated with a median time of three weeks to IRAE onset. Importantly, changes in the frequency of other circulating immune cell populations, including T cells, before and after therapy did not correlate with the development of IRAEs.

Clinical implications and future directions

Together, findings from Das and colleagues indicate that changes in circulating B cells may be useful predictors of IRAE risk (12). Clinical application of B cell monitoring could lead to earlier IRAE intervention and reduced IRAE severity, both of which would ideally translate to a reduced discontinuation of checkpoint therapy. The sample size in this study was limited, thus, a critical next step will be to determine the robustness of the proposed B cell signature in expanded patient cohorts. Significant changes in both total B cell frequency and the frequency of $CD21^{lo}$ B cells or plasmablasts were only observed in the CCB group, indicating that patients undergoing combination therapy may preferentially benefit from B cell monitoring. However, future evaluation of larger cohorts will reveal whether subsets of patients receiving monotherapy undergo similar B cell changes equally predictive of IRAE risk. It will also be necessary to determine whether changes in circulating B cells occur specifically in melanoma, or whether this signature is also detectable in patients with other tumor types.

The mechanistic contribution of B cells to IRAEs also remains unclear. While the B cell changes observed in CCB-treated patients did not correlate with the clinical response to therapy (12), it remains to be determined whether and how B cells directly mediate IRAEs. B cell receptor (BCR) sequencing of total B cells revealed post-therapy clonal expansion in a subset of patients in the CCB and monotherapy groups, but this did not correlate with the expansion of a single dominant clone, thus arguing against B cell–mediated autoreactivity against a discrete self-antigen (12). Additional studies will be required to determine the functional relevance of

$CD21^{lo}$ memory B cells and plasmablasts in IRAE pathogenesis.

Despite the outstanding questions regarding specific B cell mechanisms in IRAEs, B cell monitoring represents a relatively simple, noninvasive clinical biomarker assessment strategy that could also yield preventative benefits. Circulating biomarkers for treatment-related toxicities in other forms of immunotherapy have recently been identified and are poised to have clinical impact. For example, in cancer patients receiving chimeric antigen receptor T cell (CAR–T cell) therapy, early elevation of specific serum cytokines and other soluble factors, including IFN-γ, MIP1α, IL-6, and soluble gp130 (sgp130), precedes the development of severe cytokine release syndrome (CRS) (13, 14). Use of the IL-6 receptor (IL-6R) inhibitor tocilizumab is now approved for the treatment of severe CRS in CAR–T cell recipients, and the identification of circulating biomarkers that predict CRS may lead to prophylactic administration of tocilizumab or other cytokine inhibitors in patients who have markers associated with increased risk. Similarly, B cell changes as a biomarker for IRAEs in checkpoint inhibition therapy could lead to new preventative strategies. Along these lines, Das and colleagues suggest the potential utility of B cell–targeted therapies as a preventative measure against IRAEs. This idea is compelling, especially given the clinical success of B cell–depleting antibodies and inhibitors of Bruton's tyrosine kinase (BTK), an essential kinase for B cell maturation and signaling, for treating autoimmune diseases (15) and graft-versus-host disease (16).

It is likely that, in addition to reducing the risk of IRAEs, B cell depletion or BTK inhibition may also enhance the antitumor efficacy of checkpoint inhibitors, at least in some settings. The role of B cells in melanoma progression is controversial, as both pro- and antitumor B cell functions have been reported (17); however, the results from the use of B cell depletion in a small cohort of melanoma patients appear promising (18). Recent studies of other solid tumors have identified various B cell subsets as critical protumoral mediators of malignancy (19–25), and the enriched B cell populations identified by Das and colleagues may share similar functional properties. If so, these findings would support B cell depletion or BTK inhibition along with checkpoint inhibition as an appealing strategy to further explore. In fact, a clinical trial involving patients with head and neck squamous cell carcinoma is currently evaluating this approach (ClinicalTrials.gov NCT02454179). Time will tell whether such combinations simultaneously enhance antitumor immunity, limit IRAEs, and improve clinical outcomes. What is clear for now is that, although T cell responses are often the main focus of immunotherapy, B cells should not be overlooked.

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