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Commentary

Antibodies that target immune checkpoint molecules, such as CTLA4, provide robust antitumor effects in a subset of patients. Unfortunately, not all patients respond to immune checkpoint inhibition, and some develop life-threatening immune-related adverse events (irAEs). The mechanisms that underlie irAEs from immune checkpoint inhibition are not fully understood, and treatment strategies are currently limited to targeting inflammatory mediators. In this issue of the *JCI*, Pai et al. report on their development of a modified CTLA4 antibody that shields the inner CTLA4-binding domain until the antibody is within the protease-rich tumor microenvironment. In a lymphopenic murine model reconstituted with naive $CD4^+$ T cells, adapted anti-CTLA4 reduced the occurrence of irAEs and enhanced antitumor effects. This thought-provoking study lays the groundwork for further exploration of this adapted antibody in immunocompetent hosts and introduction of this adaptation to other immune checkpoint molecules. It also suggests that this approach may reduce the incidence of irAEs.

An adapted anti-CTLA4 therapeutic aimed at mitigating the toxicities of checkpoint inhibition

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Antibodies that target immune checkpoint molecules, such as CTLA4, provide robust antitumor effects in a subset of patients. Unfortunately, not all patients respond to immune checkpoint inhibition, and some develop life-threatening immune-related adverse events (irAEs). The mechanisms that underlie irAEs from immune checkpoint inhibition are not fully understood, and treatment strategies are currently limited to targeting inflammatory mediators. In this issue of the *JCI*, Pai et al. report on their development of a modified CTLA4 antibody that shields the inner CTLA4-binding domain until the antibody is within the protease-rich tumor microenvironment. In a lymphopenic murine model reconstituted with naive CD4$^+$ T cells, adapted anti-CTLA4 reduced the occurrence of irAEs and enhanced antitumor effects. This thought-provoking study lays the groundwork for further exploration of this adapted antibody in immunocompetent hosts and introduction of this adaptation to other immune checkpoint molecules. It also suggests that this approach may reduce the incidence of irAEs.

Limitations of immune checkpoint inhibition

Anti-CTLA4 agents are part of the flagship class of immune checkpoint inhibitors that ushered in the current era of immunotherapy for cancer. In 2011, the CTLA4 inhibitor ipilimumab was the first immune checkpoint inhibitor to be approved by the FDA (1), and in 2016, ipilimumab was approved for use in combination with the PD-1 inhibitor nivolumab (2). Together, these agents offered the first opportunity for long-term, antitumor responses for patients with advanced melanoma. Unfortunately, there are major obstacles to the use of anti-CTLA4 therapy. In particular, robust antitumor effects are limited to a subset of patients, and selected patients develop rare but serious immune-related adverse events (irAEs) (2). IrAEs are of immense clinical relevance to patients treated either with ipilimumab monotherapy, which results in 28% of patients experiencing grade 3+ irAEs, or ipilimumab and nivolumab in combination, which results in grade 3+ irAEs in up to 50% of cases (1). To date, the literature surrounding irAEs has focused on clinical descriptions characterizing the diverse manifestations and management of specific irAEs (3–5), with selected studies exploring the proposed mechanisms of irAE development (6–8). Drug development in the field of cancer immunotherapeutics has focused largely on improving antitumor efficacy. In this issue, Pai et al. report on their adaptation of an existing therapeutic to both improve efficacy and reduce the incidence of irAEs. Specifically, the authors propose a novel adaptation of the molecular structure of the anti-CTLA4 agent that maximizes antitumor effects through activity in the tumor microenvironment and ameliorates irAE development (9).

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Enhancing the good, limiting the bad

Pai and colleagues adoptively transferred naive CD4$^+$ T cells into lymphopenic *Rag1*$^{-/-}$ mice. In this model, systemic administration of anti-CTLA4 recapitulated development of irAEs, with histologically confirmed development of colitis, dermatitis, pneumonitis, and hepatitis, via proliferation of T effector cells (10) and increased production of TNF-α. In an attempt to mitigate this effect, Pai et al. subsequently engineered an anti-CTLA4 dual variable domain immunoglobulin (DVD), designed to shield the inner domain until it was within a protease-enriched tumor microenvironment. The authors postulated that this approach would maximize the effects of the anti-CTLA4 DVD on tumor-infiltrating Tregs while preserving tissue-resident Tregs, thereby limiting the exuberant inflammatory responses that result in irAEs. These findings are a reasonable explanation for the development of irAEs and are supported by published preclinical models of pneumonitis (11), colitis (12), and type I diabetes mellitus (13). Importantly, Pai and colleagues also demonstrated enhanced biodistribution of the anti-CTLA4 DVD in tumors, reduced organ toxicity, reduced activation of peripheral T effector cells, and increased antigen-specific tumor CD8$^+$ cells. Pai et al. are to be commended for approaching the mitigation of immune-related toxicity using a previously unexplored method and for providing a foundation for further work on this strategy.

Limitations and conclusions

There are however, several limitations to the study by Pai et al. The authors interrogate lymphopenic hosts and focus solely on CD4$^+$ cells, which have not been definitively shown to mediate human irAEs (14).

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Other groups have utilized human CTLA4 knockin models to interrogate irAE development in response to anti-CTLA4, anti–PD-1, or combination treatment (15). The examination of anti-CTLA4 DVD in the human CTLA4 mouse model would have strengthened the conclusions of Pai et al. In addition, results from published studies regarding the effect of anti-CTLA4 therapy on Tregs in human tumors are varied, with some data suggesting these agents may not deplete Tregs (16), but may in fact expand the Treg pool (17) or even modulate Treg-suppressive function without actually affecting numbers (18).

There are also important clinical and translational considerations that should be factored into interpretation of the findings of Pai and colleagues. Clinically, anti-CTLA4 monotherapy, as well as the combination of anti-CTLA4 and anti–PD-1, has demonstrated a survival benefit in patients with advanced melanoma (2), with combination therapy also showing an early benefit in a subset of patients with non–small cell lung cancer (19). However, anti-CTLA4 monotherapy has limited efficacy in other tumor types, potentially due to an inability to deliver higher doses, in contrast with monotherapies targeting the PD-1/PD-L1 axis, which have gained FDA approval in 13 different tumor indications to date. The DVD adaptation described by Pai et al. may thus have greater clinical impact if applied to anti–PD-1/PD-L1 agents. Moreover, the translation of a modified checkpoint inhibitor, such as anti-CTLA4 DVD, will face challenges in human cancers, including differing immunogenicity properties, antigenic heterogeneity of cancers, and the presence or absence of both specific and reliable proteases in the tumor microenvironment.

Pai et al. assert that a reduction of Tregs is the mechanism by which the anti-CTLA4 DVD will mitigate irAEs; however, the mechanisms by which irAEs develop in response to PD-1 and/or CTLA4 inhibition appear to be varied, are likely dependent on the organ-specific toxicity in question, and are unlikely to be solely mediated by Tregs. Several mechanisms of irAE development have been examined in the published literature. These include development of autoreactive T cells between both tumor and organ-specific tissues (e.g., myocarditis; ref. 20), autoantibody formation (e.g., thyroid disorders; ref. 8), cytokine-mediated toxicity (e.g., CTLA4-induced colitis; ref. 7), target tissue expression of CTLA4 (e.g., hypophysitis; ref. 21), patient germline genetics (e.g., type I diabetes mellitus; ref. 22), and gut microbiota–dependent features (e.g., protective in CTLA4 colitis; ref. 6).

Targeting inflammatory mediators is a mainstay of current clinical irAE management (23). This strategy is further supported by the finding of Pai et al. that TNF-α is markedly increased in mice receiving both anti-CTLA4 and adoptive transfer of $CD4^+$ T cells compared with untreated animals. Current guidelines suggest administration of high-dose corticosteroids for grade 3+ irAES and consideration of further immunosuppression mainly with cytokine-specific therapies, such as the TNF-α inhibitor infliximab, for steroid-refractory cases (23). Studies have shown that selective targeting of cytokines, such as with infliximab or the IL-6 inhibitor tocilizumab (23), or autoreactive T cells (Th17 cells) (24) can inhibit autoimmune effects while maintaining the antitumor benefit of therapy. Avoiding or managing toxicity using cytokine-specific targeting shows promise and would result in avoiding engineering new therapeutics as well as bypassing many of the patient-specific challenges that an anti-CTLA4 DVD would face. However, current irAE management options have proven limiting, and challenges such as infliximab-resistant toxicity do occur (4, 23). Additionally, current irAE treatment options are only effective for patients who have or are about to experience an irAE. They do not prevent the irAE from developing or associated immune-mediated organ damage. With the known inability to prevent irAEs in mind, modification of the therapeutic itself, as Pai et al. suggest, gains merit.

In summary, Pai et al. offer a thought-provoking article that examines a new therapeutic strategy by which the benefits of checkpoint inhibition may be maximized, while the off-target effects of these agents, largely through a biologic basis of altering Tregs, are minimized. Further study in this area could focus on examining these effects in immunocompetent models, the effects on other potential mechanisms of immune-related toxicity, and application of this approach to other checkpoint molecules.

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