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# Anti-PD-1 antibodies for the treatment of B-cell lymphoma

## Importance of PD-1+ T-cell subsets

Fuliang Chu and Sattva S Neelapu*

[1]Department of Lymphoma and Myeloma; The University of Texas M.D. Anderson Cancer Center; Houston, TX USA;
[2]Center for Cancer Immunology Research; The University of Texas M.D. Anderson Cancer Center; Houston, TX USA

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Monoclonal antibodies specific for programmed cell death 1 (PDCD1, best known as PD-1) have been shown to mediate antineoplastic effects in follicular lymphoma patients. However, the relative proportion of intratumoral PD-1+ T-cell subsets, in particular follicular helper T cells (which exert pro-tumor functions) and effector T cells (which have anticancer activity), may impact clinical outcome, and should therefore be carefully considered for patient selection in this setting.

The blockade of immune checkpoints with monoclonal antibodies specific for programmed cell death 1 (PDCD1, best known as PD-1) induces major clinical responses in a significant proportion of patients with solid malignancies including melanoma, lung cancer, and renal cell carcinoma,[1,2] but its efficacy against hematological cancers is unknown. Recently, we reported the results of a Phase 2 clinical trial in which we evaluated the efficacy of an anti-PD-1 monoclonal antibody in patients with follicular lymphoma,[3] the most common indolent B-cell non-Hodgkin lymphoma worldwide. This hematological neoplasm originates from germinal center B cells and is generally considered incurable.

In this setting, patients with relapsed follicular lymphoma were treated with a combination of pidilizumab, a humanized monoclonal IgG1κ specific for PD-1 and rituximab, a chimeric monoclonal IgG1κ targeting CD20.[3] Pidilizumab was administered at 3 mg/kg intravenously for up to 12 infusions at 4-wk intervals. Rituximab was started approximately 2 wk after the first infusion of pidilizumab and was administered i.v. at the standard dose of 375 mg/m$^2$ body surface area, on a weekly schedule, for 4 wk. The primary endpoint was overall response rate (ORR) and the trial was powered to detect a 20% improvement in ORR as compared with historical results of rituximab monotherapy, which is associated with 40% ORR when used as retreatment.[4] The combination therapy was safe, provoking no autoimmune or Grade 3/4 adverse events. Nineteen out of 29 evaluable patients manifested an objective clinical response, making up an ORR = 66%. The observed complete response rate (52%) was markedly superior to that expected with rituximab monotherapy (11%).[4] Furthermore, after a median follow-up of 15.4 mo, the median progression-free survival (PFS) for all patients was 18.8 mo and was not reached for the 19 responders.[3]

Analysis of paired peripheral blood and tumor samples by flow cytometry and gene expression profiling, respectively, at baseline and 14 d after the first infusion of pidilizumab revealed the activation of T and natural killer (NK) cells in both compartments. More interestingly, high expression levels of CD274 (best known as PD-L1) but not PD-1 or PDCD1 ligand 2 (PDCD1LG2, best known as PD-L2) on circulating CD4+ and CD8+ T cells at baseline were associated with improved clinical response.[3] Though we could not determine whether the expression levels of PD-L1 in the peripheral blood and tumor microenvironment correlate with each other, the former are likely to constitute a surrogate marker for the latter, for at least 2 reasons. First, autologous antitumor T cells could readily be isolated from the peripheral blood of follicular lymphoma patients, suggesting that they circulate between the tumor and peripheral blood.[5] Second, we have documented the expression of PD-L1 on tumor-infiltrating T cells in follicular lymphoma patients (FC and SSN, unpublished observations). The expression of PD-L1 by malignant cells has been suggested as a marker of endogenous antitumor immunity, reflecting the phenomenon of immune escape induced by interferon γ (IFNγ) and possibly other cytokines that are secreted by antitumor effector T cells (Teffs).[6] The exposure of intratumoral T cells to the cytokine milieu to which malignant cells are normally exposed might therefore result in the expression of PD-L1. If our findings are confirmed in large patient cohorts, the expression of PD-L1 on circulating T cells might serve as a novel biomarker that is easily assessable by flow cytometry, providing an

*Correspondence to: Sattva S Neelapu; Email: sneelapu@mdanderson.org
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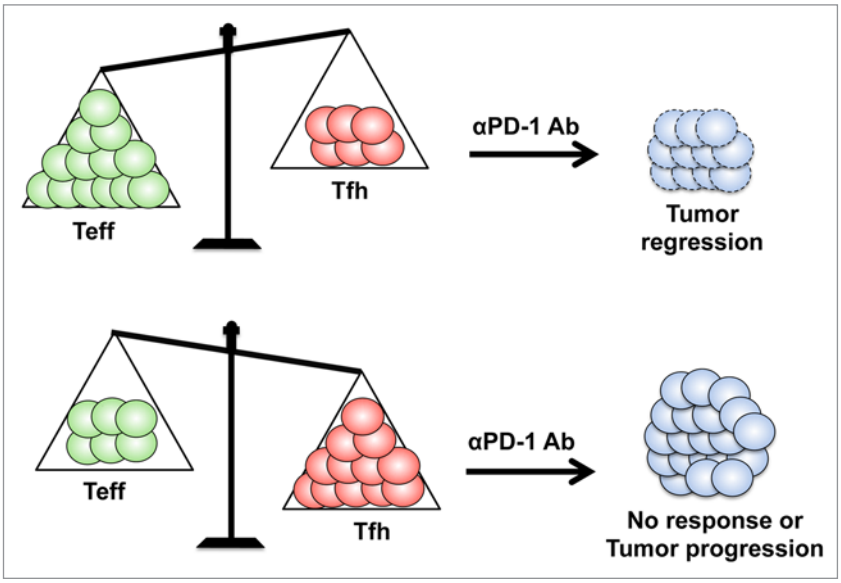


**Figure 1.** Impact of PD-1$^+$ T-cell subsets on clinical outcome after therapy with anti-PD-1 antibody in follicular lymphoma. Patients with higher numbers of PD-1$^{int}$ or PD-1$^{lo/-}$ effectors T cells (Teffs) relative to PD-1$^{hi}$ follicular helper T cells (Tfh) likely have tumor regression after anti-PD-1 antibody (Ab) therapy (top panel). In contrast, patients with higher numbers of PD-1$^{hi}$ Tfh relative to PD-1$^{int}$ or PD-1$^{lo/-}$ Teffs likely have either no response or tumor progression after anti-PD-1 antibody therapy (bottom panel).

alternative to the immunohistochemical evaluation of PD-L1 expression on tumor biopsies.[1,6]

Although our and other studies have pointed to PD-L1 expression levels as a potential biomarker of response to anti-PD-1 antibodies, the relative proportion of multiple pro- and antitumor PD-1$^+$ T-cell subsets in the tumor microenvironment may also impact clinical outcome. Within follicular lymphomas, at least 4 distinct T-cell subsets express PD-1: follicular helper T (Tfh) cells and follicular regulatory T (Tfr) cells, both of which express high levels of PD-1, as well as (CD4$^+$ and CD8$^+$) Teffs and non-Tfr regulatory T cells (Tregs), both of which express PD-1 to intermediate levels.[7-9] Of these T-cell subsets, Tfh cells and non-Tfr Tregs are likely to mediate tumor-supporting effects, while Teffs and Tfr cells presumably exert an antitumor activity.[5,7-10] Blocking PD-1 enhances the antitumor functions of Teffs but the effect of this intervention on other PD-1$^+$ T-cell subsets is unknown. We found that a 41-component gene signature that is expressed at high levels by the Teffs (and low levels by the Tfh cells) of the follicular lymphoma microenvironment is associated with clinical outcome.[3] When this signature is overexpressed in baseline tumor biopsies, follicular lymphomas contain increased amounts of Teffs relative to Tfh cells, a settting that is associated with improved clinical responses and superior PFS (**Fig. 1**). Notably, this signature did not correlate with clinical outcome in an independent cohort of follicular lymphoma patients treated with chemotherapy, suggesting that its predictive value may be limited to the response to anti-PD-1 antibodies. If confirmed in larger studies, our findings would raise the intriguing possibility that blocking PD-1 may enhance the function of both pro-tumor Tfh cells and antitumor Teffs, suggesting that the relative proportion of these T-cell subsets as well as that of PD-1$^+$ Tregs should be considered (in addition to PD-L1 expression levels) to optimally select patients for anti-PD-1 antibody-based therapy. Perhaps this applies not only to follicular lymphoma patients but also to individuals affected by other malignancies. The evaluation of higher doses of pidilizumab and different administration schedules may further enhance the efficacy of this immunotherapeutic regimen.

In conclusion, our results suggest that anti-PD-1 antibodies are safe and effective in follicular lymphoma patients. However, clinical outcome may depend on the unique composition of the tumor microenvironment, in particular the relative proportion of distinct PD-1$^+$ T-cell subsets.

### Disclosure of Potential Conflicts of Interest

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