

IN THE SPOTLIGHT

Another LAP in the Race

Sylvia M. Lee^{1,2} and Cassian Yee^{1,2}

Summary: The unique promise of latency-associated peptide resides in its selective presence on regulatory T cells (Treg) in the activated setting after patients are treated with immunomodulators such as anti-CTLA-4. The improved ability to track, scrutinize, and potentially target Tregs in this manipulated environment will be increasingly critical in developing immune-based therapies for patients with cancer. *Cancer Discovery*; 2(2): 107-9. ©2012 AACR.

Commentary on Sun et al., p. 122 (7).

The identification of regulatory T cells (Treg), long thought to be mythical objects of immunologic hand-waving, led to a greater appreciation for the agility of the immune response and the mechanisms responsible for initiating and tempering a cell-mediated response to pathogens, autoantigens, and tumor cells. From the start, however, there have always been limitations to the markers that were used to identify such Tregs.

In 1995, the laboratory of Sakaguchi first described a population of CD4⁺ T cells constitutively expressing elevated levels of CD25 on the surface that exhibited immunosuppressive properties (1). Expression of FoxP3 was later linked to this hallmark feature of Tregs—suppression of a proliferative or functional lymphocyte response—and subsequently became the phenotypic gold standard for a Treg (2).

In the last decade, Tregs were found to play a prominent role in tumor immunity and immune escape; not only was an increased frequency of Tregs in peripheral blood and at tumor sites associated with disease stage and progression in patients with cancer (3, 4), but the ratio of Tregs to other effector cells appeared to hold prognostic significance (5). In addition, it was demonstrated that the depletion of Tregs could induce tumor eradication in animal models (6).

Thus, the race goes on to characterize Tregs, to elucidate their mechanisms and relationships with other cells, and to understand what factors control their expansion and contraction. However, the ability to study Tregs in a more precise and nondestructive fashion has been hampered by the specificity (or lack thereof) of known markers. Although high surface CD25 expression is commonly used to identify Tregs, recent activation of effector T cells can lead to upregulation of CD25 as well, confounding the identification of true Tregs *in vivo* and *in vitro*. The most widely accepted marker for Tregs, FoxP3, is found intracellularly; therefore, fixation and

permeabilization are required to detect expression, limiting the ability to isolate viable T cells for downstream studies or to target Tregs using surface ligands (Fig. 1).

Other surface markers, such as CD127, which is found to be decreased or absent among CD4⁺CD25^{hi} T cells, have recently been used to attain a more refined population of Tregs. However, as Sun and colleagues (7) demonstrate in their article in this issue of *Cancer Discovery*, this combination also fails to adequately distinguish a functionally suppressive Treg population from conventional effector T cells in the activated setting.

In vitro activation of effector CD4 T cells leads to a CD127^{lo}, CD25^{hi} phenotype not dissimilar from the natural Treg, thus confounding nondestructive identification of Tregs. With the advent of cell-cycle checkpoint inhibitors to the clinical arena, such as the recently approved anti-CTLA-4 antibody ipilimumab, the intrinsic CTLA-4 brake on T-cell proliferation can be released after T-cell receptor engagement, leading to sustained activation and unchecked proliferation of effector T cells; for tumor immunologists, this is a desirable perturbation leading to enhancement of the endogenous T-cell responses to tumor-associated antigens. However, global activation leads to upregulation of CD25 and lowered CD127 levels among activated effectors, recapitulating a similar phenotype observed after *in vitro* activation. Thus, distinguishing these “beneficial” antitumor effectors from the population of Tregs on the basis of CD25 and CD127 expression in this setting can be problematic.

In recent years, there has been growing interest in the role of latency-associated peptide (LAP) and its presence on the surface of a subset of Tregs. LAP binds and forms a latent complex with TGF- β , one of the principal cytokines by which Tregs are thought to suppress immune responses. In 2008, Chen and colleagues (8) described a unique population of CD4⁺CD25⁺LAP⁺ Tregs that demonstrated more potent suppressive activity that was TGF- β dependent compared with CD4⁺CD25⁺LAP⁻ Tregs. LAP also was identified by Tran and colleagues (9) to be a new marker that could help distinguish activated Tregs from activated FoxP3⁻ and FoxP3⁺ T effectors.

In this issue of *Cancer Discovery*, Sun and colleagues (7) demonstrate that although CD25^{hi}, CD127^{lo} is a good marker for unactivated Tregs both phenotypically (correlation with FoxP3) and functionally (maintenance of suppressive

Authors' Affiliations: ¹Clinical Research Division, Fred Hutchinson Cancer Research Center and ²Department of Medicine, University of Washington, Seattle, Washington

Corresponding Author: Cassian Yee, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Mailstop D3-100, Seattle, WA 98109. Phone: 206-667-6287; Fax: 206-667-7983; E-mail: cyee@fhcrc.org

doi: 10.1158/2159-8290.CD-12-0010

©2012 American Association for Cancer Research.

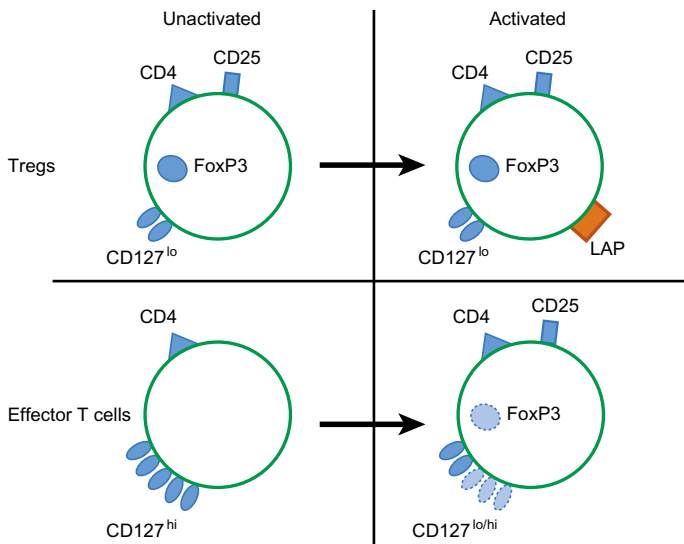


Figure 1. Currently used markers for Tregs do not adequately distinguish Tregs from effector T cells in the activated setting. The expression of CD25, FoxP3, and low CD127 can be seen on both Tregs and activated effector T cells. Furthermore, because FoxP3 is an intracellular marker, its detection relies on the destruction of the T cell, preventing the isolation of viable Treg populations for functional studies or *ex vivo* expansion. In contrast, LAP provides a surface marker that can accurately distinguish Tregs from effector T cells after both *in vitro* activation and after treatment with anti-CTLA-4 immunotherapy, without compromising the integrity of the cell.

capacity), the same cannot be said for healthy donor peripheral blood mononuclear cells after *in vitro* activation, where more than two thirds of such CD25^{hi}, CD127^{lo} cells lacked suppressive capacity. The group, however, was able to demonstrate a positive correlation with FoxP3 expression and functional suppression among the population of LAP⁺ cells, which had increased in frequency from 0.3% to 12% after *in vitro* activation.

Along similar but even more compelling lines, when samples were collected from patients after anti-CTLA-4 therapy, CD4⁺CD25⁺CD127^{lo} cells from posttherapy samples were incapable of suppressing lymphocyte proliferation, whereas CD4⁺CD25⁺LAP⁺ T cells were functionally suppressive. Therefore, LAP positivity more accurately reflects the Treg population among patients receiving anti-CTLA-4 therapy, and potentially, patients treated with other immunomodulatory-based therapies as well, such as inhibitors of the PD1/PDL-1 axis checkpoint (10).

For anti-CTLA-4 and likely for other immune-based strategies in which the clinical response is often delayed for weeks or months, sometimes worsening before improving, the identification of a predictive marker would be a valuable clinical tool (11). This new era of ipilimumab and its novel response patterns has presented oncologists with unfamiliar treatment dilemmas in which they are forced to decide, with very little radiographic guidance and in the face of possible autoimmune toxicities, whether to discontinue potentially promising therapy. The use of LAP alone or in combination with other markers (e.g., inducible costimulator; refs. 12, 13), may allow the formulation of a predictive algorithm to aid in clinical decision-making.

The greater precision afforded by LAP in selecting Tregs may also provide a new strategy for Treg depletion. *In vivo* depletion of Tregs by the use of agents such as denileukin diftitox (interleukin-2-conjugated diphtheria toxin) or dalizumab (anti-CD25 Ab) that target CD25 expression have led to occasional successes (14) but sometimes inconsistent results (15, 16). The development of novel targeted agents against LAP could provide a more effective means of achieving Treg depletion, albeit with a potential new set of toxicities

from the on-target damage to LAP⁺ megakaryocytes, platelets, and immature dendritic cells.

As the early success of checkpoint inhibitors ignites an explosion of research into additional applications and combination strategies (e.g., PD1/PDL1, ipilimumab in conjunction with vaccines, ipilimumab, and BRAF inhibition), the ability to accurately track Tregs in these manipulated environments will become increasingly important. Sun and colleagues (7) make the additional point that LAP and FoxP3 expression are not perfectly concordant on CD4⁺CD25⁺ T cells (i.e., there are T cells that are LAP⁺ but FoxP3⁻ and other T cells that are LAP⁻ but FoxP3⁺). With growing evidence that points to the remarkable heterogeneity of Tregs, it remains to be seen how LAP expression will play out in the future delineation of Treg subpopulations, including the more recently discovered CD8⁺ Tregs. Ongoing efforts by this group and others to identify and characterize Treg subsets, and to identify reliable markers for following Tregs under clinically relevant conditions, will keep us on track to developing more effective immunotherapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Received January 9, 2012; accepted January 9, 2012; published online February 13, 2012.

REFERENCES

1. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995;255:1151-64.
2. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057-61.
3. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, et al. Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res* 2001;21:4766-72.
4. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma

- fosters immune privilege and predicts reduced survival. *Nat Med* 2004;20:942–9.
5. Bui JD, Uppaluri R, Hsieh CS, Schreiber RD. Comparative analysis of regulatory and effector T cells in progressively growing versus rejecting tumors of similar origins. *Cancer Res* 2006;26:7301–9.
 6. Yu P, Lee Y, Liu W, Krausz T, Chong A, Schreiber H, et al. Intratumor depletion of CD4+ cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors. *J Exp Med* 2005;201:779–91.
 7. Sun J, Tang DN, Fu T, Sharma P. Identification of human regulatory T cells in the setting of T-cell activation and anti-CTLA-4 immunotherapy on the basis of expression of latency-associated peptide. *Cancer Discovery* 2012;2:122–30.
 8. Chen ML, Yan BS, Bando Y, Kuchroo VK, Weiner HL. Latency-associated peptide identifies a novel CD4+CD25+ regulatory T cell subset with TGFbeta-mediated function and enhanced suppression of experimental autoimmune encephalomyelitis. *J Immunol* 2008;280:7327–37.
 9. Tran DQ, Andersson J, Hardwick D, Bebris L, Illei GG, Shevach EM. Selective expression of latency-associated peptide (LAP) and IL-1 receptor type I/II (CD121a/CD121b) on activated human FOXP3+ regulatory T cells allows for their purification from expansion cultures. *Blood* 2009;213:5125–33.
 10. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, et al. Sharfman WH. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* 2010;28:3167–75.
 11. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res* 2009;25:7412–20.
 12. Chen H, Liakou CI, Kamat A, Pettaway C, Ward JF, Tang DN, et al. Anti-CTLA-4 therapy results in higher CD4+ICOShi T cell frequency and IFN-gamma levels in both nonmalignant and malignant prostate tissues. *Proc Natl Acad Sci U S A* 2009;206:2729–34.
 13. Liakou CI, Kamat A, Tang DN, Chen H, Sun J, Troncoso P, et al. CTLA-4 blockade increases IFN-gamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients. *Proc Natl Acad Sci U S A* 2008;205:14987–92.
 14. Rech AJ, Vonderheide, RH. Clinical use of anti-CD25 antibody daclizumab to enhance immune responses to tumor antigen vaccination by targeting regulatory T cells. *Ann NY Acad Sci* 2009;2174:29–106.
 15. Dannull J, Su Z, Rizzieri D, Yang BK, Coleman D, Yancey D, et al. Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. *J Clin Invest* 2005;215:3623–33.
 16. Attia P, Maker AV, Haworth LR, Rogers-Freezer L, Rosenberg SA. Inability of a fusion protein of IL-2 and diphtheria toxin (Denileukin Diftitox, DAB389IL-2, ONTAK) to eliminate regulatory T lymphocytes in patients with melanoma. *J Immunother* 2005;28:582–92.