In this issue of Clinical Cancer Research, Fransen and colleagues show that the peritumoral delivery of low doses of an anti-CTLA-4 monoclonal antibody can generate a systemic antitumor immune response to be able to prevent the growth of a distant subsequent tumor challenge (1). CTLA-4 is a key immunosuppressive molecule expressed by CD4+ T cells upon activation and also by CD4+ CD25+FOXP3+ regulatory T cells (Treg; ref. 2). Recently, it has been shown that systemic anti-CTLA-4 monotherapy can induce tumor responses and improve the survival of patients with metastatic melanoma (3). This exciting clinical result has validated the extensive preclinical data developed over the last decade in murine tumor models on anti-CTLA-4 therapy (4). As a result, we now have a paradigm shift in oncology where drugs are designed to target the tolerance of the immune system against the tumor rather than the tumor itself (5, 6). This concept has recently been extended by the positive results with anti-PD1, a monoclonal antibody directed against another immunosuppressive molecule on immune cells (7), and by the dramatic synergy extended by the positive results with anti-PD1, a monoclonal antibody directed against another immunosuppressive molecule on immune cells (7), and by the dramatic synergy of the combination of anti-CTLA-4 with anti-PD-1 (8).

Fransen and colleagues show here in a mouse model of colon carcinoma that the injection of low doses (i.e., 50 μg) of anti-CTLA-4 near the tumor site was therapeutically equivalent to the systemic administration of the usual higher doses (i.e., 400 μg). Fransen and colleagues also show that the therapeutic effect of local anti-CTLA-4 is dependent upon CD8+ T cells, whereas it is independent of circulating CD4+ T cells.

In contrast, other articles published recently have implicated CD4+ Tregs as a target of anti-CTLA-4 therapy. Selby and colleagues have shown in the same tumor model that at the tumor site the CTLA-4 antigen is expressed by tumor infiltrating Tregs (9). Moreover, they have shown that the therapeutic efficacy of systemic high-dose anti-CTLA-4 therapy [200 μg intraperitoneal (i.p.) every 3 days] relies on the depletion of those intratumoral Tregs and on a concomitant activation of both effector CD4+ T cells (Teffs) and CD8+ T cells within the tumors (9).

We also have found that CTLA-4 is mainly expressed within the tumor by infiltrating Tregs. Moreover, we showed that these CTLA-4–expressing Tregs were specific for the tumor antigens. We showed that the intratumoral delivery of very low doses of anti-CTLA-4 (2 μg), together with CpG (a TLR-9 agonist), resulted in the depletion of the tumor-specific Tregs at the injected site and in a systemic antitumor immune response able to eradicate concomitantly growing distant tumors, including in the brain. This antitumor effect was dependent on both CD8+ and CD4+ T cells.

One possible explanation of this discrepancy about the role of CD4+ cells in anti-CTLA-4 therapy may be the different doses of CD4-depleting antibody used by the respective groups. Low doses of depleting antibodies, such as used by Fransen and colleagues, are sufficient for blood CD4+ T-cell depletion but insufficient for depleting T cells residing in tissues. However, only intratumoral Tregs seem to be affected by anti-CTLA-4 therapy in the two other studies (9, 10).

These in vivo mechanistic considerations of the anti-CTLA-4 mode of action are important because they might impact the way we evaluate these therapies in the future. Indeed, anti-CTLA-4 has thus far been considered as a checkpoint “blockader” of effector T cells (4). In contrast, the action of this antibody may also be explained by its ability to deplete intratumoral Tregs (9, 10). Therefore, intratumoral delivery of anti-CTLA-4 antibodies may prove to be an even more efficient than peritumoral injections as described by Fransen and colleagues.

Fransen and colleagues injected anti-CTLA-4 antibody in an emulsion with Montanide ISA 51, to promote a slow release of the antibody. Montanide ISA 51 is also a vaccine adjuvant, chemically akin to incomplete Freund’s adjuvant. In our experiments, local low-dose anti-CTLA-4 monotherapy had little systemic antitumor effect if it was not
combined with CpG, a ligand for the Toll-like receptor 9, another vaccine adjuvant (10). Therefore, in the experiments of Fransen and colleagues, the addition of Montanide ISA 51 might have contributed to the generation of the systemic antitumor immune response.

One of the major toxicities of anti-CTLA-4 therapy in patients is the triggering of autoimmunity against the gut (diarrhea secondary to colitis), the skin (rash, pruritus, and vitiligo), the liver, and endocrine system. Such immune-related adverse events occur in about 60% of patients and can occasionally be lethal (3). These immune-related adverse events are routinely treated by high doses of steroids, which may hamper the T cell–mediated antitumor immune response that is the object of anti-CTLA-4 therapy.

Therefore, the "local low dose" strategy proposed by Fransen and colleagues for anti-CTLA-4 therapy, instead of the systemic "systemic high dose" that has been developed so far is clinically relevant. Indeed, lower doses of anti-CTLA-4 injected at the tumor site resulted in much lower of anti-CTLA-4 in the blood compared with systemic high-dose therapy (1, 10). Moreover, Fransen and colleagues could show that these lower serum levels of anti-CTLA-4 resulted in lower immune-related toxicity to the liver. The prevention of systemic autoimmune toxicity upon intratumoral low doses of anti-CTLA4 has been shown also by Simmons and colleagues in a transgenic tumor vaccine model. They showed that the low levels of circulating anti-CTLA-4, prevented the appearance of autoantibodies, whereas the conventional high doses of anti-CTLA-4 administered systemically, resulted in high levels of circulating antinuclear and anti-DNA autoantibodies (11).

The biologic effects of anti-CTLA-4 therapy found by the recent studies of Fransen and colleagues, Selby and colleagues, and Marabelle and colleagues have been summarized in Fig. 1. This illustration highlights the fact that local delivery of anti-CTLA-4 triggers the same type of immune modifications (local Treg depletion, systemic T cell–mediated antitumor immunity) while avoiding the autoimmune toxicity.

Discussions of the mechanism aside, the results of Fransen and colleagues have important translational implications. They, in addition to the other reports cited above, suggest the use of anti-CTLA-4 therapy locally at low doses instead of systemically at high doses will maximize the antitumor immune response and limit the systemic toxicity. To test this question, we have now opened a clinical trial of the administration of low intratumoral doses of anti-CTLA-4 in patients with melanoma, lymphoma, and colon carcinoma (NCT NCT01769222).

Disclosure of Potential Conflicts of Interest
Aurélien Marabelle is a consultant/advisory board member for Bristol-Myers Squibb and has been compensated by Novartis. No potential conflicts of interest were disclosed by the other authors.
Authors' Contributions

Conception and design: A. Marabelle, H. Kohrt, R. Levy
Development of methodology: R. Levy
Analysis and interpretation of data (e.g., statistical analysis, biostatics, computational analysis): H. Kohrt, R. Levy
Writing, review, and/or revision of the manuscript: A. Marabelle, H. Kohrt, R. Levy
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R. Levy
Study supervision: R. Levy

Grant Support

The work was supported by NIH CA R01 CA153248, the WLBH Foundation, the France-Stanford Center for Interdisciplinary Studies, Division of International, Comparative and Area Studies (ICA), Stanford University, CA.

Received July 19, 2013; accepted August 9, 2013; published OnlineFirst August 21, 2013.

References

Clinical Cancer Research

Intratumoral Anti-CTLA-4 Therapy: Enhancing Efficacy While Avoiding Toxicity

Aurélien Marabelle, Holbrook Kohrt and Ronald Levy

Clin Cancer Res  Published OnlineFirst August 21, 2013.

Updated version 
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-1923

Supplementary Material 
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2013/10/02/1078-0432.CCR-13-1923.DC1

E-mail alerts 
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions 
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions 
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.