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

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SUMMARY

Systemic administration of the checkpoint blockade antibody anti-CTLA4 results in severe auto-immune toxicity, limiting its clinical efficacy. Fransen and colleagues show here that peri-tumoral delivery of low doses of this immunomodulatory drug can trigger a systemic anti-tumor immune response while preventing the toxicity against other organs.

COMMENTARY

In this issue of Clinical Cancer Research, Fransen and colleagues show that the peri-tumoral delivery of low doses of an anti-CTLA-4 monoclonal antibody can generate a systemic anti-tumor immune response 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 (Tregs) (2). Recently, it has been demonstrated 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 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. 50µg) of anti-CTLA-4 near the tumor site was therapeutically equivalent to the systemic administration of the usual higher doses (i.e. 400µg). Fransen et al 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.

By contrast, other papers published recently have implicated CD4 positive Tregs as a target of anti-CTLA-4 therapy. Selby and colleagues have demonstrated in the same tumor model that at the tumor site the CTLA-4 antigen is expressed by tumor infiltrating Tregs. Moreover they have shown that the therapeutic efficacy of systemic high dose anti-CTLA-4 therapy (200µg i.p. every 3 days) relies on the depletion of those intra-tumoral Tregs and on a

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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 demonstrated that these CTLA-4 expressing, Tregs were specific for the tumor antigens. We showed that the intra-tumoral delivery of very low doses of anti-CTLA-4 (2 μ g), together with CpG (a TLR-9 agonist), resulted in the depletion of the tumor-specific Tregs at the injected site and in a systemic anti-tumor immune response able to eradicate concomitantly growing distant tumors, including in the brain. This anti-tumor 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 et al., are sufficient for blood CD4⁺ T-cell depletion but insufficient for depleting T-cells residing in tissues. However, only intra-tumoral Tregs seem to be affected by anti-CTLA4 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). By contrast, the action of this antibody may also be explained by its ability to deplete intra-tumoral Tregs (9,10). Therefore intra-tumoral delivery of anti-CTLA-4 antibodies may prove to be an even more efficient than peri-tumoral injections as described by Fransen et al.

Fransen et al 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 anti-tumor effect if it was not combined with CpG, a ligand for the Toll Like Redepor 9, another vaccine adjuvant (10). Therefore, in the experiments of Fransen et al. the addition of Montanide ISA 51 might have contributed to the generation of the systemic anti-tumor immune response.

One of the major toxicities of anti-CTLA-4 therapy in patients is the triggering of auto-immunity against the gut (diarrhea secondary to colitis), the skin (rash, pruritus, 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 anti-tumor immune response that is the object of anti-CTLA-4 therapy.

Therefore the “local low dose” strategy proposed by Fransen et al 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 to systemic high dose therapy (1,10). Moreover, Fransen et al could show that these lower serum levels of anti-CTLA-4 resulted in lower immune related toxicity to the liver. The prevention of systemic auto-immune toxicity upon intra-tumoral low doses of anti-CTLA4 has been demonstrated also by Simmons et al in a transgenic tumor vaccine model. They showed that the low levels of circulating anti-CTLA-4, prevented the appearance of auto-antibodies, whereas the conventional high doses of anti-CTLA-4 administered systemically resulted in high levels of circulating anti-nuclear and anti-DNA auto-antibodies (11).

The biological effects of anti-CTLA-4 therapy found by the recent studies of Fransen et al, Selby et al and Marabelle et al have been summarized in Figure 1. This illustration highlights the fact that local delivery of anti-CTLA-4 triggers the same type of immune modifications (local T-reg depletion, systemic T-cell mediated anti-tumor immunity) while avoiding the auto-immune toxicity.

Discussions of the mechanism aside, the results of Fransen et al 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 anti-tumor immune response and limit the systemic toxicity. In order 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).

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REFERENCES

1. Fransen MF, Van der Sluis TC, Ossendorp F, Arens R, Melief CJ. Controlled local delivery of CTLA-4 blocking antibody induces CD8+ T cell dependent tumor eradication and decreases risk of toxic side-effects. *Clin Cancer Res*. 2013
2. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. *Science*. 2008; 322:271–5. [PubMed: 18845758]
3. Hodi FSS, O'Day SJSJ, McDermott DFDF, Weber RWRW, Sosman JAJA, Haanen JBJB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363:711–23. [PubMed: 20525992]
4. Quezada SA, Peggs KS, Simpson TR, Allison JP. Shifting the equilibrium in cancer immunoediting: from tumor tolerance to eradication. *Immunol Rev*. 2011; 241:104–18. [PubMed: 21488893]
5. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature*. 2011; 480:480–9. [PubMed: 22193102]
6. Topalian SL, Weiner GJ, Pardoll DM. Cancer immunotherapy comes of age. *J Clin Oncology*. 2011; 29:4828–36.
7. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012; 366:2443–54. [PubMed: 22658127]
8. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus Ipilimumab in Advanced Melanoma. *N Engl J Med*. 2013; 369:122–33. [PubMed: 23724867]
9. Selby MJ, Engelhardt JJ, Quigley M, Henning KA, Chen T, Srinivasan M, et al. Anti-CTLA-4 Antibodies of IgG2a Isotype Enhance Antitumor Activity through Reduction of Intratumoral Regulatory T Cells. *Cancer Immunology Research*. 2013; 1:32.
10. Marabelle A, Kohrt H, Sagiv-Barfi I, Ajami B, Axtell RC, Zhou G, et al. Depleting tumor-specific Tregs at a single site eradicates disseminated tumors. *J Clin Invest*. 2013; 123:2447–63. [PubMed: 23728179]
11. Simmons AD, Moskalenko M, Creson J, Fang J, Yi S, VanRoey MJ, et al. Local secretion of anti-CTLA-4 enhances the therapeutic efficacy of a cancer immunotherapy with reduced evidence of systemic autoimmunity. *Cancer Immunol Immunother*. 2008; 57:1263–70. [PubMed: 18236040]

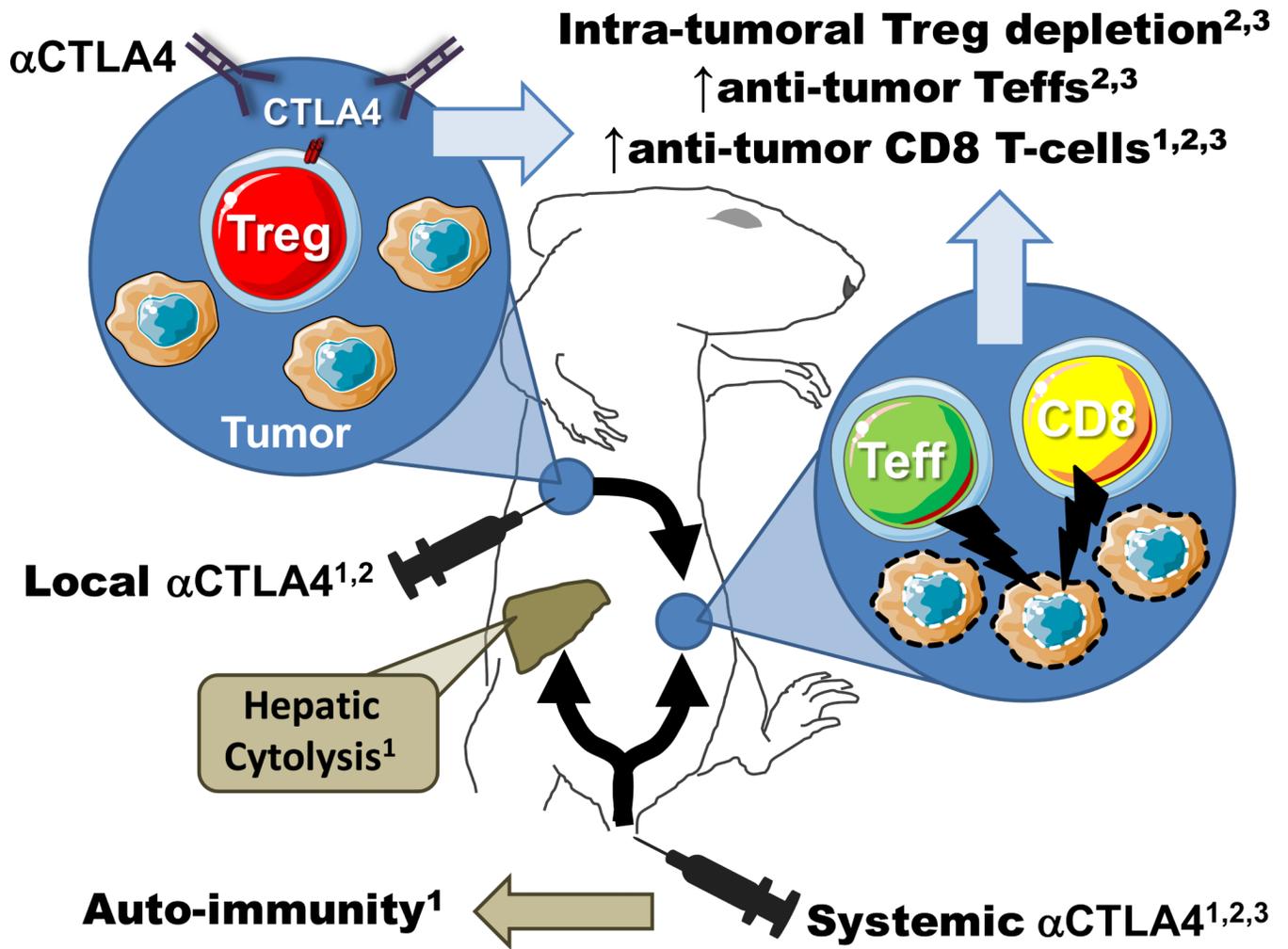


Figure.
 Graphical summary of the main results from Fransen et al (1), Marabelle et al (2) and Selby et al (3) on the effect of anti-CTLA-4 therapy. Intra or peri-tumoral injections of anti-CTLA-4 trigger a CD4 and CD8 mediated anti-tumor immune response able to eradicate tumor cells at a distant site. The use of low doses of anti-CTLA-4 avoids the auto-immune toxicity of a systemic high dose delivery.