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Intratumoral checkpoint subversion as a strategy for minimizing adverse effects

Harvesting the power of TILs without harvesting TILs

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Anti-CTLA4 Therapy: Benefits and Risks

One of the most significant clinical advances in cancer immunotherapy to date has been the targeting of the immune "checkpoints" that inhibit effector T-cell function. Cytotoxic T lymphocyte-associated protein 4 (CTLA4), which was one among the first checkpoint regulators to be characterized, is transiently expressed by activated effector T cells and constitutively expressed on regulatory T cells (Tregs).[1] CTLA4 inhibits T cells by at least 2 mechanisms: (1) by preferentially binding CD80 or CD86 on antigen-presenting cells (APCs), thereby antagonizing the co-stimulatory signals delivered to T cells via CD28;[2] and (2) by sending an anergy-inducing signal via the intracellular kinases phosphoinositide-3-kinase (PI3K) and AKT1 (also known as protein kinase B, PKB).[3] Systemic CTLA4 blockade confers antitumor immunity in murine models[4] and improves the overall survival of melanoma patients,[5] but is associated with dose-dependent and occasionally fatal autoimmune toxicities, including pneumonitis, enterocolitis, and hepatitis[6]. An approach that maximizes the activation of antitumor T cells while keeping T lymphocytes specific for self structures at bay would increase the benefit-to-risk ratio of this form of immunotherapy and provide immediate advantage to cancer patients.

Local Anti-CTLA4 Therapy, Could it Work?

Recent studies by Marabelle et al.[7] and Fransen et al.[8] demonstrate that the *local* delivery of anti-CTLA4 antibodies at low doses can be as effective at eliciting antitumor immune responses as the *systemic* delivery of the same antibodies at standard doses. The mechanisms underlying the improved therapeutic profile of this approach warrant further in-depth investigation. At least theoretically, the blockade of CTLA4 at one tumor site is expected to enable *local* antitumor immunity by allowing for CD28-dependent co-stimulation and by preventing the delivery of an anergy-promoting signal. However, once these T cells migrate to a *distant* tumor, the blockade on CTLA4 should be relieved, CD28 signaling prevented and tumor-specific anergy perpetuated. By contrast, *if* the local blockade of CTLA4 were to induce some irreversible modifications in tumor-infiltrating lymphocytes (TILs), making them resistant to immunosuppression, this would promote the establishment of systemic antitumor immunity. There is significant experimental data in support of the latter hypothesis. TILs, when harvested and aggressively activated ex vivo, are indeed capable of overcoming the immunosuppressive microenvironment of distant tumors, thereby mediating robust antitumor responses.[9]

The question addressed by Marabelle et al. and Fransen et al. is whether (and how) the in vivo manipulation of TILs at one tumor site might induce a similarly potent activation and render them resistant to immunosuppression (**Fig. 1**). The local blockade of CTLA4 rendered effector T cells resistant to the immunosuppressive activity of Tregs in vitro.[10,11] If this finding extended to an in vivo setting, then the blockade of CTLA4 at one "priming" tumor site would render T cells insensitive to Treg-dependent immunosuppression at distant sites. Additional signals could be required to achieve such a priming, and activated APCs might constitute a rich source of these signals, including T_H1-priming cytokines such as interleukin (IL)-12 and IL-18, we well as co-stimulatory ligands like CD80, CD86 and tumor necrosis factor (ligand) superfamily, member 4 (TNFSF4, best known as OX40 ligand). For example, it has been shown that the ligation of tumor necrosis factor receptor superfamily, member 4 (TNFRSF4, best known as OX40) by agonist antibodies disables Treg[12] and downregulates the expression of CTLA4 on effector T cells.[13] Presumably, these OX40-targeting antibodies mimic the signal normally provided by the OX40L molecules expressed by activated dendritic cells (DCs). Similarly, lipid-based adjuvants such as Montanide[14] have been shown to enhance the activation of cytotoxic T lymphocytes (CTLs),[15] and recruit T cells as well as $CD83^{high}$ DCs[16] in both pre-clinical studies and early phase clinical trials.[17] $CD83^{high}$ DCs might prime TILs in a variety of ways, including the inhibition of the membrane-associated ring finger (C3HC4) 1 (MARCH1)-dependent ubiquitination and subsequent degradation of MHC class II molecules and CD86.[18]

Local Anti-CTLA4 Therapy Can Boost Anti-Tumor Immunity – Recurring Themes

In a murine lymphoma model, Marabelle et al. demonstrated that the intratumoral injection of CpG oligodeoxynucleotides (ODNs) together with

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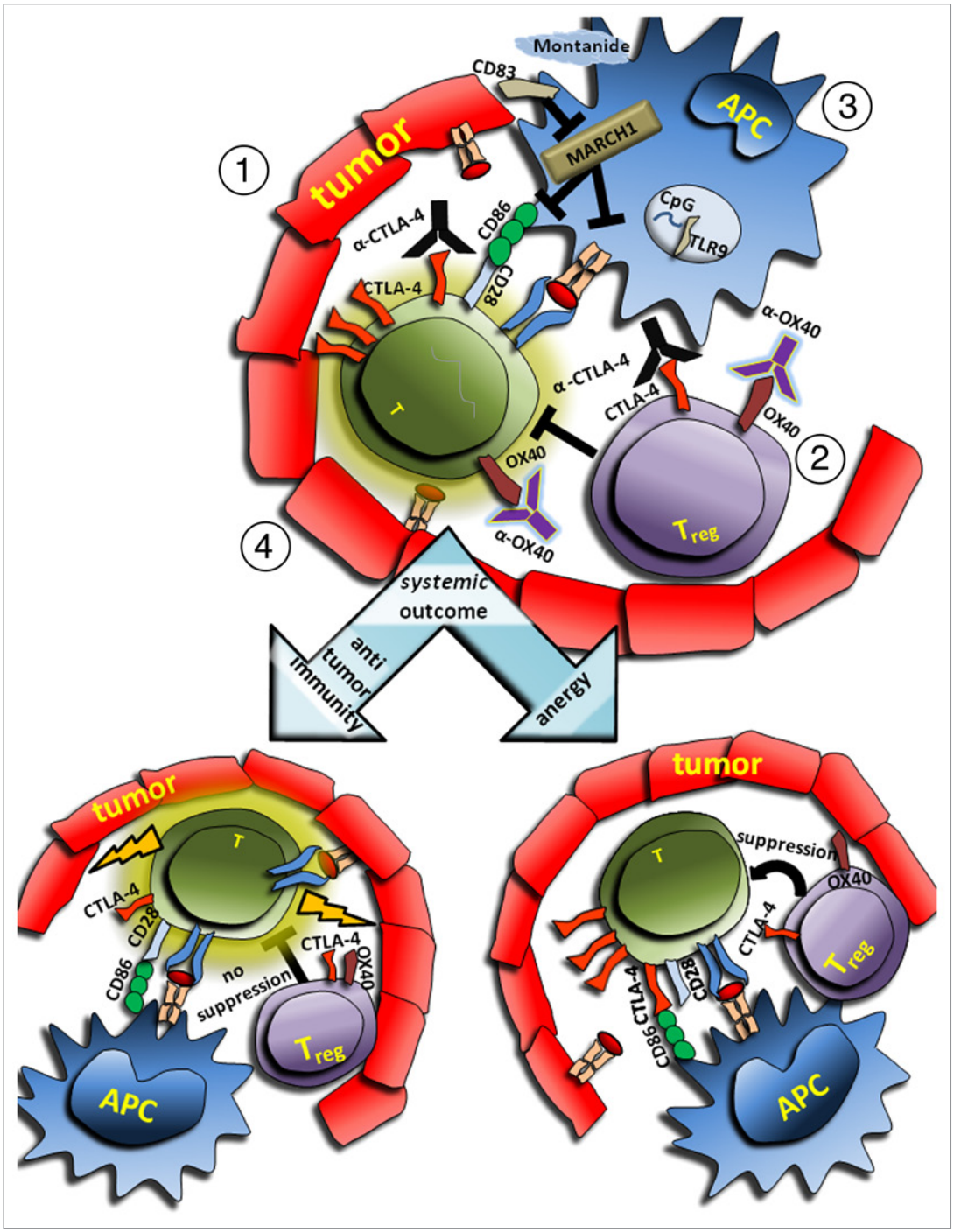

Figure 1. Mechanisms by which local T-cell modulation may overcome anergy to achieve systemic antitumor immunity. **(1)** Anti-CTLA4 antibodies induce a transient resistance of T cells to regulatory T cell (Treg)-mediated immunosuppression. **(2)** Depleting Tregs or converting them into effector T cells with anti-CTLA-4 and anti-OX40 antibodies may relieve the numerous signals by they repress the activity of tumor-infiltrating lymphocytes (TILs), including interleukin (IL)-4, IL-10, IL-13, IL-35 and transforming growth factor β1 (TGFβ1). **(3)** Stimulating the recruitment and activation of antigen-presenting cells (APCs) with adjuvants like CpG oligodeoxynucleotides or Montanide can results in a $MHCII^{high}CD86^{high}$ phenotype through multiple pathways, including the CD83-dependent inhibition of membrane-associated ring finger (C3HC4) 1 (MARCH1). **(4)** Anti-OX40 antibodies can mimic the activity of OX40 ligand (OX40L) molecules expressed on activated APCs, decreasing the expression levels of CTLA4 on TILs. TLR9, Toll-like receptor 9.

low doses of anti-CTLA4 and anti-OX40 antibodies mediates antineoplastic effects that are superior to those elicited by the systemic administration of anti-CTLA4 antibodies at a 100-fold higher dose. In contrast to systemic therapy, this maneuver generates robust immunological memory and confers long-term protection against tumor challenges. Similarly, in a murine colon carcinoma model, Fransen et al. showed that the peritumoral injection of low doses of an anti-CTLA4

antibodies in Montanide induces systemic antitumor immunity as effectively as the systemic administration of the same molecules at standard doses. In both models, the induction of $CD8^+$ T cells specific for a tumor-associated antigen (i.e., ovalbumin) and the dependence of antitumor immunity upon the $CD8^+$ T-cell population as a whole were demonstrated. Additionally, both studies showed that the local delivery of low-dose anti-CTLA4 antibodies yields significantly (i.e., 1000-fold) lower levels of serum antibodies than the systemic administration, and that this immunotherapeutic paradigm is effective against other tumor types (i.e., breast adenocarcinoma and T-cell lymphoma). These pre-clinical findings have prominent clinical implications given the dose-dependent side effects documented in cancer patients receiving anti-CTLA4 antibodies systemically.

Local anti-CTLA4 Therapy is Not Enough – Model-Dependent Differences

Though both the studies by Marabelle et al. and Fransen et al. demonstrated that the local administration of anti-CTLA4 antibodies at low doses exerts antineoplastic effects, the co-delivery of CTLA4-targeting antibodies with Montanide was as effective as the systemic approach, while the combination of anti-CTLA4 antibodies with CpG ODNs and anti-OX40 antibodies achieved superior efficacy as compared with the systemic administration. The studies also highlighted notable mechanistic differences. Marabelle et al. demonstrated indeed that the intratumoral injection of anti-CTLA4 antbiodies, anti-OX40 antibodies and CpG ODNs depletes tumor-resident Tregs (including tumor-specific Trefs), a process that is accompanied by the accumulation of both $CD4^+$ and $CD8^+$ effector T cells that mediate anticancer immune responses. By contrast, Fransen et al. showed that local delivery of CTLA4-targeting antibodies induces a tumor-specific immune response that relies upon $CD8^+$ but not $CD4^+$ cells. These discrepancies may originate from the therapeutic regimens or reflects variables in the model system employed, including tumor type or injection site (*i.e.*, intratumoral vs. peritumoral). Favoring the former possibility, another group has shown that the intratumoral delivery of anti-CTLA4 antibodies did not deplete tumor-resident Tregs, but only did so when combined with anti-CD25 antibodies.[19] These findings suggest that the local blockade of CTLA4 as a stand-alone immunotherapeutic intervention is insufficient to induce systemic immune responses against all tumor types.

Local Anti-CTLA4 Treatment in the Clinic

The promising pre-clinical results and practical elegance of these novel efforts to increase both the efficacy and safety of anticancer immunotherapy have prompted several clinical trials testing variations thereof. A study of intratumoral ipilimumab combined with local radiotherapy in patients with melanoma, lymphoma, or colorectal cancer (NCT01769222) has recently been initiated in Stanford (California). This approach may harness the capacity of radiotherapy to promote the exposure of the endoplasmic reticulum protein calreticulin to the cell membrane and induce immunogenic cell death.[20] An alternate approach being evaluated at the Huntsman Cancer Institute (Salt Lake City, Utah) is the intratumoral injection of interleukin-2 (IL-2) plus ipilimumab in patients with unresectable melanoma (NCT01672450). In this setting, targeting CTLA4 might counteract the proliferative effect of high local IL-2 concentrations on Tregs[21] or skew the microenvironment toward the proliferation of TILs, hence inducing systemic antitumor immune responses. The results of this study may serve as an interesting counterpoint to a related approach currently being investigated in Tübingen (Germany). In this setting, intratumoral IL-2 is combined with systemic ipilimumab (at standard dosing) (NCT01480323). Cumulatively, these studies will allow for assessing the safety and efficacy of IL-2 at intratumoral doses ranging from 3.3% to 67% of standard systemic regimens.

Overall, the translation-minded rationale and impressive pre-clinical science behind these studies represent a meaningful progress in the field of applied oncoimmunology. This progress sits atop our decision tree of possible immunotherapeutic combinations, weighing down the branches and bringing some fruitful results within reach, which will be real, near-term benefits to cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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