Intratumoral Immunization: A New Paradigm for Cancer Therapy

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Review

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Intratumoral Immunization: A New Paradigm for Cancer Therapy

Aurélien Marabelle[1], Holbrook Kohrt[2], Christophe Caux[1], and Ronald Levy[2]

Abstract

Immune cell infiltration in the tumor microenvironment is of prognostic and therapeutic import. These immune cell subsets can be heterogeneous and are composed of mature antigen-presenting cells, helper and effector cytotoxic T cells, toleragenic dendritic cells, tumor-associated macrophages, and regulatory T cells, among other cell types. With the development of novel drugs that target the immune system rather than the cancer cells, the tumor immune microenvironment is not only prognostic for overall patient outcome, but also predictive for likelihood of response to these immune-targeted therapies. Such therapies aim to reverse the cancer immunotolerance and trigger an effective antitumor immune response. Two major families of immunostimulatory drugs are currently in clinical development: pattern recognition receptor agonists (PRRago) and immunostimulatory monoclonal antibodies (ISmAb). Despite their immune-targeted design, these agents have so far been developed clinically as if they were typical anticancer drugs. Here, we review the limitations of this conventional approach, specifically addressing the shortcomings of the usual schedules of intravenous infusions every 2 or 3 weeks. If the new modalities of immunotherapy target specific immune cells within the tumor microenvironment, it might be preferable to deliver them locally into the tumor rather than systemically. There is preclinical and clinical evidence that a therapeutic systemic antitumor immune response can be generated upon intratumoral immunomodulation. Moreover, preclinical results have shown that therapeutic synergy can be obtained by combining PRRagos and ISmAbs to the local tumor site. *Clin Cancer Res; 20(7); 1747–56. ©2014 AACR.*

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CME Staff Planners' Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

Upon completion of this activity, the participant should have a better understanding of the rationale for immunostimulation in cancer therapy and the evidence-based combination strategies of intratumoral immunization currently in preclinical and clinical development.

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Introduction

Major efforts have been made over the past several decades to develop cytotoxic drugs that specifically target cancer cells. Many of these drugs have resulted in tumor responses and improved overall survival. However, many patients are primarily refractory to these tumor-targeted therapies or develop relapse with tumor subclones that do not have the therapeutic target and are therefore resistant to the therapy. This phenomenon has been well illustrated in patients with metastatic melanoma who initially have dramatic responses to the BRAF inhibitor vemurafenib and then quickly relapse with tumors that are resistant to BRAF inhibition (1).

Recently, therapies have been designed to specifically target the immune system rather than cancer cells. The aim of these new drugs is to interact with molecules playing a role in the activation of immune cells to reverse the cancer-

Authors' Affiliations: [1]Centre de Recherche en Cancérologie de Lyon, UMR INSERM U1052 CNRS 5286, Centre Léon Bérard, Université de Lyon, Lyon, France; and [2]Division of Oncology, Stanford University, Department of Medicine, Stanford, California

Corresponding Author: Aurelien Marabelle, Léon Bérard Cancer Center, 28 rue Laennec, 69008 Lyon, France. Phone: 33-4-6916-6595; Fax: 33-4-7878-2709; E-mail: aurelien.marabelle@lyon.unicancer.fr

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induced immunotolerance and allow an antitumor immune response to occur. This principle has recently been proven by the positive results of clinical trials of these new therapies in metastatic melanoma, renal cell carcinoma, and non–small cell lung cancer (NSCLC), diseases with low sensitivity to conventional cytotoxic therapies. The consequence of these positive results is a paradigm shift in oncology in which the clinical problem of cancer may be considered not only to be the accumulation of genetic abnormalities in the tumor cells, but also the tolerance of these abnormal cells by the immune system.

Two families of new drugs that are directed at the immune system include pattern recognition receptor agonists (PRRago) and immunostimulatory monoclonal antibodies (ISmAb). Immune cells expressing the targets of these new drugs are present within the tumor microenvironment. Interestingly, evidence is accumulating to support the idea that these new drugs work by targeting intratumoral immune cells. Therefore, as opposed to conventional anticancer drugs, these immunostimulatory drugs can be delivered directly into the tumor, even at a single site, and generate a systemic antitumor immune response. This intratumoral delivery can trigger even more potent antitumor immune responses while causing less autoimmune toxicity. Interestingly, in preclinical models only certain combinations of immunomodulatory agents are additive or synergistic in their therapeutic effects and induce curative systemic antitumor immunity. Here, we review the evidence for the effectiveness of intratumoral immunization.

Reversing Tumor Tolerance and Boosting the Antitumor Immune Response by Targeting Intratumoral Pattern Recognition Receptors

Pattern recognition receptors (PRR) constitute a constantly growing family of receptors having the ability to recognize pathogen-associated molecular patterns (PAMP) such as bacterial cell wall molecules or viral DNA, and damage-associated molecular patterns (DAMP) released upon cell death, stress or tissue injury. Toll-like receptors (TLR), a subfamily of PRRs, are highly expressed by immune cells from both myeloid and lymphoid lineages that infiltrate the tumor microenvironment, such as tumor-associated macrophages (TAM), plasmacytoid and myeloid dendritic cells (pDC and mDC), $CD4^+$ and $CD8^+$ T cells, regulatory T cells (Treg), natural killer (NK) cells, and B cells (Table 1). The pattern and level of expression of TLRs can vary depending on the immune cell lineage subsets (e.g., mDCs subsets) and their state of activation (e.g., upon B-cell receptor stimulation; refs. 2, 3). The level of infiltration of some of these cells has a prognostic value in many cancer types (Table 2).

The negative prognostic value of tumor-infiltrating macrophages, tumor-associated dendritic cells (DC), and Tregs can be explained by their ability to inhibit antitumor immune responses (4). Indeed, hematocytotoxic conditioning (chemotherapy or total body irradiation) that depletes these cells has enhanced the efficacy of antitumor adoptive T-cell therapy (5).

Upon stimulation by their ligands, TLRs trigger the activation of the host cells [notably antigen-presenting cells (APC)] and the secretion of proinflammatory cytokines such as type I IFNs, interleukin (IL)-6 and IL-12. This mechanism plays a role in the activation of immune responses against infectious pathogens. Now there is a clear demonstration that TLR activation by PAMPs and DAMPs also plays a role in immune responses against tumor cells. Indeed, TLR stimulation of APCs within mice and in the human tumor microenvironment modifies their phenotype from tolerogenic to immunogenic, with the upregulation of class II MHC, CD80, and CD86 (6, 7). Such activation of APCs is a prerequisite to sustain the development of an efficient adaptive antitumor immune response.

TLRs can also be expressed by tumor cells. The direct activation of TLRs on cancer cells can result in the death of the targeted tumor cell and/or, for B-cell lymphomas, upregulate antigen presentation molecules (8, 9). Moreover, upon chemotherapy or tumor-targeted therapy, tumor cells can release endogenous TLR agonists (DAMPs), which can stimulate the immune cells surrounding the tumor cells. This phenomenon has been well illustrated with HMGB1, an intracellular protein released in the tumor milieu upon

Table 1. Immunostimulatory targets on tumor-infiltrating human immune cells

Cell types	PRRago targets	ISmAb targets
pDCs	TLR-7, 9, 10	PD-L1, CD137
mDCs	TLR-1/2, 3, 4, 5, 2/6, 8	PD-L1, CD137
Macrophages	TLR-1/2, 4, 5, 2/6, 8	PD-L1
$CD8^+$ T cells	TLR-5, 8	PD-1, PD-L1, CD137, CTLA-4^{low}
Activated $CD4^+$ T cells (including Tregs)	TLR-5, 8	OX40, CD137, PD-1, CTLA-4
B cells	TLR-1/2, 7/8, 9, 10	CD137, PD-1
NK cells	TLR-1/2, 5	KIR, CD137, PD-1
Tumor cells	+/−TLRs	PD-L1

Abbreviations: CD137, also known as 4-1BB; KIR, killer immunoglobulin-like receptors; PD-1, programmed cell death 1; PD-L1, PD-1 ligand; OX40, also known as CD134.

Table 2. Diversity of cancer types with prognostic immune contexture

Tumor-infiltrating immune cells	Prognostic value in	Reference
DCs	Ovarian cancer	(57)
	Breast cancer	(58)
	Colon cancer	(59)
	Lung cancer	(60)
	Oral SCC	(61)
	Melanoma	(62)
	Gastric cancer	(63)
	Gallbladder carcinoma	(64)
TAMs	Neuroblastoma	(65)
	Osteosarcoma	(66)
	Breast cancer	(67)
	Ewing sarcoma	(68)
Tregs	NSCLC	(69)
	Pancreatic cancer	(70)
	Gastric cancer	(71)
	Hepatocellular carcinoma	(72)
	Ovarian carcinoma	(73)
$CD8^+$ T cells	Colon cancer	(74)
	NSCLC	(75)
	Ovarian cancer	(76)
	Melanoma	(77)

NOTE: Tumor infiltration by DCs, TAMs, and Tregs is usually associated with a bad prognosis, whereas high levels of $CD8^+$ T cells are classically correlated with a better clinical outcome. However, this generality is controversial because some series have found opposite results for some cancer types. These controversies should be solved in the future when refined techniques will allow us to determine the activation status and the antigen specificity of these immune cells and their proportion in precise areas within the tumor microenvironment.

tumor cell death and which is subsequently recognized by TLR-4 expressed on tumor-infiltrating immune cells. The demonstration that TLR activation happens upon tumor cell death and that it is a key factor of response to conventional therapies has led to the concept of immunogenic cell death as opposed to tolerogenic cell death (10). However, in some cases, TLR stimulation alone might also have a prooncogenic effect and stimulate the proliferation of cancer cells; see recent review in this journal (11).

Intratumoral immune stimulation can also be obtained by targeting intratumoral RIG-I–like receptors (RLR). RLRs are another PRR subfamily historically considered to be sensors of virus double-stranded RNA upon viral infection. Upon stimulation by their ligands, RLRs trigger the release of type I IFNs by the host cell and eventually result in its death by apoptosis (12). Such cytokine and tumor-associated antigen (TAA) release can also result in the activation of the antitumor immune response (13). As opposed to TLRs, RLRs are endogenously expressed in all tumor cell types, making them a universal proimmunogenic therapeutic target (14). The stimulation of RLRs should be of particular relevance in the immune response generated upon intratumoral delivery of oncolytic viruses.

Using Tumors as Their Own Vaccines: Intratumoral Delivery of PRRagos in Human Cancers

Tumor responses upon intratumoral delivery of pathogens have been described since the end of the 19th century. Dr. William Coley, a surgeon at what would later become Memorial Sloan-Kettering Cancer Center (New York, NY), turned the phenomenon into a medical practice. He confirmed that intratumoral injections of extracts from bacteria responsible for erysipelas (*Streptococcus pneumoniae* and *Serratia marcescens*) could cure solid tumors (15). Later, accumulating preclinical evidence supported the use of Bacillus Calmette–Guerin (BCG) for cancer therapy (16). Clinicians reported the therapeutic benefits of intratumoral injections of BCG in several types of cancer, such as melanoma (17–20) or squamous cell carcinoma (SCC) of the head and neck (21). The University of Texas MD Anderson Cancer Center (Houston, TX) reported up to 2,500 patients with all types of cancer treated with BCG, including scarification of the tumors (22). Interestingly, Morton and colleagues reported that in patients with metastatic melanoma, intratumoral injections of BCG induced regressions in about 90% of the injected tumor sites and in about 20% of the distant, uninjected tumor sites (18). Bast and colleagues reviewed 12 studies of intratumoral BCG in patients with metastatic cutaneous melanoma and found that injected tumors showed regression in 58% of the cases, and that distant, noninjected tumor sites showed regression in 14% of the cases (23). Shimada and colleagues identified that the therapeutic effects of BCG were partly due to the proinflammatory properties of the nucleic acid fraction of BCG (24). Indeed, the ability of BCG DNA and cell wall skeleton to activate PRRs explains many of its immunostimulatory properties (25, 26). Interestingly, local delivery of PRRago molecules seems to be as efficient as live bacteria injections to induce local control of tumors. Topical imiquimod has 70% to 90% clearance rates in superficial skin cancers such as basal cell carcinomas and SCC (27). In a phase I/II study of cutaneous melanoma, topical imiquimod was able to induce a 40% rate of complete responses with or without intralesional IL-2 (28). Imiquimod in combination with intralesional BCG was able to induce complete remission in 5 of 9 patients with cutaneous melanoma (29).

Intratumoral PRRagos can also generate some levels of systemic antitumor immunity inducing tumor responses in distant, uninjected, tumor sites. Repeated intratumoral CpG (PF-3512676) at one single tumor site together with a 2×2 Gy local irradiation was able to induce an overall response rate of 27% in distant untreated sites of patients with metastatic follicular lymphoma (9). The ability to

generate distant tumor responses upon local injections of a PRRago was subsequently confirmed with the same therapy in 5 of 15 patients with metastatic cutaneous T-cell lymphoma (7). The ability of intratumoral PRRagos to generate a systemic antitumor immune response has also been studied in preclinical models. In mice, as in humans, intratumoral PRRagos usually trigger a local cytotoxic antitumor immune response that can result in complete regression of the injected tumor, but that has limited effect on the distant, uninjected tumor sites (8, 30).

Mode of Action of Therapeutic Intratumoral PRRagos

The local delivery of these immunostimulatory drugs is supported by the fact that many cells of the tumor microenvironment express PRRs (Table 1). The mechanism of intratumoral PRRagos therapeutic effect is multifactorial, depending on the tumor cell type, the tumor microenvironment, and the PRRago used. For instance, CpG, a TLR9 agonist, will have a direct cytotoxic effect against TLR9-positive B-cell lymphoma tumor cells, but will also stimulate the antigen-presenting ability of the remaining tumor B cells, thereby helping the generation of an antitumor immune response (8, 31). The cytokines released upon CpG injections have been shown to induce in an antigen nonspecific manner a transient helper phenotype to Tregs, stimulating antigen cross-presentation and priming of cytotoxic $CD8^+$ T cells via the expression of CD40L (32). Imiquimod, a TLR7 agonist, has a therapeutic effect when applied on subcutaneous mouse melanoma tumors mediated by a direct killing of tumor cells by pDCs via a TRAIL/DR5 and granzyme B mechanism and independently of adaptive immune cells (33). Shime and colleagues have demonstrated that PolyI:C, a TLR3 agonist, could convert tumor-supporting macrophages into tumoricidal effectors in a mouse model of lung carcinoma (34).

A common feature can be found between all the PRRagos used in therapy though. All of them should have a stimulating effect on tumor-infiltrating APCs (B cells, DCs, TAMs, and other myeloid derived suppressor cells) mediated by proinflammatory cytokine secretion and upregulation of costimulatory molecules on their surface. Indeed, preclinical results have recently demonstrated in mice that intratumoral delivery of PRRagos stimulates the antitumor immune response via the activation of APCs infiltrating the tumors (high expression of MHC II, CD80, and CD86; refs. 6, 8). This common feature is a prerequisite for mounting an efficient adaptive antitumor immune response against TAAs, but it does not address efficiently the issues of immunosuppressive tumor-infiltrating Tregs and anergic/exhausted tumor infiltrating or peritumoral cytotoxic T cells (35).

Breaking Tumor Tolerance and Boosting Antitumor Immune Response by Targeting Intratumoral Checkpoint Molecules

In oncology, ISmAbs are designed to target specifically molecules involved in the regulation of the immune system with the aim of reversing tumor immunotolerance and stimulating antitumor immune response. Many of them are currently in clinical development (Table 3; ref. 36). Interestingly, these checkpoint molecules have been described to be highly expressed by immune cells infiltrating the tumor microenvironment (Table 1).

The most clinically advanced of these new ISmAbs is the antagonistic anti–CTLA-4 ipilimumab (Yervoy; Bristol-Myers Squibb), which is approved by the U.S. Food and Drug Administration and the European Medicines Agency for the treatment of metastatic melanoma. In two subsequent randomized phase III clinical trials, systemic intravenous therapy with ipilimumab generated long-lasting tumor responses in up to 20% of patients with refractory/relapsing melanoma (37, 38). However, this therapy was associated with major autoimmune toxicities requiring high-dose steroids in about 60% of the patients treated. Anti–CTLA-4 antitumor efficacy has been so far explained by the ability of this antagonistic monoclonal antibody to block the inhibitory interaction of CTLA-4 expressed on effector T cells with CD80/86 expressed by tolerogenic tumor APCs.

Interestingly, recent data suggest that the *in vivo* efficacy of antagonistic anti–CTLA-4 therapy might be due to an intratumoral depletion of Tregs rather than an interaction with $CD4^+$ effector T cells (39). Indeed, intratumoral tumor–specific Tregs express high levels of CTLA-4 and are depleted upon therapy with anti–CTLA-4 via $Fc\gamma R^+$ tumor–infiltrating cells (40–42). These results can explain the systemic antitumor immune response that can be generated in mouse models with only local low-dose delivery of anti–CTLA-4. Fransen and colleagues demonstrated recently that low doses of anti–CTLA-4 delivered into a water-in-oil emulsion adjuvant (Montanide ISA51) around an established mouse colon carcinoma tumor was able to eradicate the local tumor and prevent the development of tumors at a distant noninjected site (43). Interestingly, this intratumoral Treg depletion also explains the *in vivo* efficacy of agonistic antibodies targeting the costimulatory molecules GITR and OX40 (40, 42). These results open a new perspective on the mechanism of action of these ISmAbs and emphasize the importance of their design, especially their isotype.

Systemic Tumor Responses upon Intratumoral Immunomodulation

In humans, rare observations of systemic tumor responses upon local irradiation have been reported historically and are referred to as bystander effects or the "abscopal" effect (44). The incidence of this abscopal effect seems to be potentiated when local irradiation is combined with an immune modulatory strategy. As mentioned above, local irradiation combined with intratumoral CpG generates tumor responses in distant sites in patients with metastatic follicular B-cell lymphoma and cutaneous T-cell lymphoma (7, 9). Observations of abscopal effects have also been described upon combination of local irradiation

Table 3. Immunostimulatory mAbs currently in clinical development

Therapeutic molecule	Name	Sponsor	Ongoing trials
Anti-CD137 (4-1BB)	PF-05082566	Pfizer	NCT01307267
	Urelumab (BMS-663513)	BMS	NCT01471210
			NCT01775631
Anti-CD134 (OX40)	Anti-OX40 antibody	Providence Health and Services	NCT01642290
			NCT01862900
			NCT01303705
Anti–PD-1	Nivolumab (MDX 1106/BMS-936558/ONO 4538)	BMS	NCT01658878
			NCT01629758
			NCT01176461
			NCT01968109
			NCT01714739
			NCT01592370
			NCT01673867
			NCT01721746
			NCT01721772
			NCT01668784
			NCT01844505
			NCT01642004
	Pidilizumab (CT-011)	Curetech	NCT01441765
			NCT01096602
			NCT01067287
			NCT01952769
			NCT01313416
	MK-3475/SCH 900475	Merck/Schering Plough	NCT01295827
			NCT01840579
			NCT01905657
			NCT01866319
			NCT01848834
			NCT01876511
			NCT01953692
	MEDI4736	Medimmune/Astra Zeneca	NCT01938612
			NCT01693562
			NCT01975831
Anti-KIR	Lirilumab/BMS-986015	BMS	NCT01714739
			NCT01750580
			NCT01714739
Anti–LAG-3	BMS-986016	BMS	NCT01968109
Anti–PD-L1	MSB0010718C	Merck KGaA/EMD Serono	NCT01943461
			NCT01772004
	MPDL3280A	Roche/Genentech	NCT01846416
			NCT01633970
			NCT01903993
			NCT01375842
			NCT01656642
Anti–CTLA-4	Tremelimumab	Medimmune/Astra Zeneca	NCT01975831
			NCT01843374
			NCT01853618
			NCT01103635
	Ipilimumab	BMS	>80 trials
Anti-CD40	CP-870,893		NCT01456585
			NCT01103635

and systemic anti–CTLA-4 immunomodulation in patients with metastatic melanoma (45–47).

Distant effects have also been observed upon oncolytic virus therapy. These viruses have been genetically modified for better tumor cell selectivity and expression of immunostimulatory cytokines such as granulocyte macrophage colony-stimulating factor (GM-CSF), IL-12, or type I IFN. Although not yet clearly defined, due to their pathogen structure all these viruses should also have PRRago properties from their capside proteins or internal nucleic acids. For instance, DNA virus can be turned into dsRNA and subsequently activate RLRs (48). Interestingly, intratumoral delivery of such viruses is able to generate a systemic antitumor immune response. Intratumoral JX-594/TG6006 oncolytic virus in 14 patients with primary liver tumors or metastatic intrahepatic nodules was able to induce partial responses (−30% to −50% in diameter) of both injected and distant tumor sites (49). These findings have been subsequently confirmed in another randomized phase II study in patients with hepatocellular carcinoma in whom the same disease control was obtained in injected and distant sites (50). Many intratumoral immunization clinical trials are currently ongoing, using intratumoral immunostimulatory products with the aim of generating a systemic antitumor immune response (Table 4).

Preclinical models have recently demonstrated that the efficacy of immunostimulatory drugs is potentiated upon intratumoral injections. The hypothesis behind such a practice is that by delivering locally high concentrations of immunomodulatory drug, we could trigger a more efficient antitumor immune response. Dubrot and colleagues showed that intratumoral injection of type I IFN alone or anti-CD137 systemic therapy alone has little therapeutic effect against the MC38 mouse colon carcinoma (51). However, the combinations of intratumoral IFN-α together with systemic high-dose anti-CD137 synergize and generate immune-mediated tumor responses at distant noninjected sites. Subsequently, the same team showed in the same colon carcinoma model that intratumoral low doses of anti-CD137 (5 μg i.t. instead of 100 μg i.p./injection) injected into one tumor site was sufficient to eradicate both injected and distant noninjected sites in 50% of the mice (52). This therapeutic effect was additive to the therapeutic effect of systemic anti–PD-L1 therapy, and the combination of intratumoral anti-CD137 + systemic anti–PD-L1 was able to cure most of the mice. Most importantly, intratumoral injections of low-dose anti-CD137 avoided autoimmune hepatocytolysis and liver T-cell infiltration that is generated by the same drug when administered systemically. As was the case for anti-OX40 and anti-GITR, local anti-CD137 effect could also be mediated via intratumoral Treg depletion because Tregs also express high levels of CD137. Intratumoral injections of anti-CD137 and an engineered IL-2Fc fusion protein anchored to the surface of PEGylated liposomes avoided systemic toxicity (weight loss and high cytokine circulating levels) while eliciting local and systemic antitumor immunity (53). However, in this model, the systemic antitumor immune response was weak as it only slowed the tumor growth of distant sites. Besides the difference of the tumor model (B16 melanoma instead of MC38), this anti-CD137 + IL-2 strategy might not be optimal at generating a potent systemic antitumor immune response due to the stimulatory properties of IL-2 on Tregs (54).

Fransen and colleagues showed that for the same antitumor efficacy, liver enzymes were lower upon local low-dose anti–CTLA-4 rather than for a systemic high dose (43). Simmons and colleagues also demonstrated that local immunomodulation with a transgenic melanoma tumor cell vaccine delivering GM-CSF and anti–CTLA-4 *in situ* was able to generate systemic antitumor immunity while preventing the rise of circulating levels of autoimmunity markers (ANA, ssDNA, and dsDNA) happening upon prolonged anti–CTLA-4 therapy (55). The lower toxicity of local low-dose immunomodulation versus systemic high dose is of course explained by much lower circulating doses of ISmAbs in the blood of recipients (40, 43, 55).

Interestingly, a potentiation of immunomodulatory drugs can also be observed upon intratumoral combinations. A triple combination of intratumoral CpG, together with low doses of anti-OX40 and anti–CTLA-4 (100-fold lower doses than usual systemic doses), is sufficient to trigger a systemic CD4 and CD8 T-cell–mediated antitumor immune response able to eradicate distant metastatic tumor sites, including metastases in the central nervous system in almost all mice treated. This local combination strategy generated a better $CD8^+$ memory antitumor immune response because it prevented late tumor relapses as opposed to systemic delivery of ISmAbs. This therapeutic combination was less effective with a dual combination of CpG and low-dose ISmAb and was not effective at all if CpG was injected outside the tumor (40). The fact that a triple combination does better than a double is at least partly due to the additive effect on the ability of these drugs to deplete intratumoral Tregs. The requirement of having CpG coinjected into the tumor can be explained by recent results showing that the *in vivo* therapeutic effects of ISmAbs via Treg depletion probably relies on antibody-dependent cell-mediated cytotoxicity (ADCC; refs. 41, 42). The fact that CpG stimulates ADCC might explain why it potentiates Treg depletion upon combination with ISmAbs (56). Together, these data suggest that to generate an efficient systemic adaptive antitumor immune response, intratumoral immunization strategies should combine Treg depletion with immunogenic tumor cell death and APC activation (Fig. 1).

Practical and Clinical Consequences of Local Delivery of Immunostimulatory Drugs

Local delivery of immunostimulating drugs should prevent their circulation at high concentrations in the blood. Moreover, local injections allow much higher concentrations of the immunostimulatory products in the tumor

Table 4. Ongoing intratumoral immunization trials

Trial design	Trial sponsor	Disease	Trial no.
IT Ipilimumab (anti–CTLA-4) and local radiotherapy	Stanford University	B-, T-, and NK-cell lymphomas Colon and rectal cancers Melanoma	NCT01769222
IT Ipilimumab (anti–CTLA-4) and IT IL-2	University of Utah	Metastatic melanoma	NCT01672450
IT IL-2 and IV Ipilimumab (anti–CTLA-4)	University Hospital Tuebingen	Metastatic melanoma	NCT01480323
IT Talimogene laherparepvec transgenic oncolytic virus expressing GM-CSF and IV ipilimumab (anti–CTLA-4)	Amgen	Metastatic melanoma	NCT01740297
IT Poly-ICLC TLR3 agonist and IT Flt3L cytokine and local radiotherapy	Mount Sinai School of Medicine	Low-grade B-cell lymphoma	NCT01976585
IT Electroporation of IL-12 plasmid	OncoSec Medical Inc.	Cutaneous T-cell lymphomas Mycosis fungoides Merkel carcinoma	NCT01579318 NCT01502293 NCT01440816
IT Alpha-Gal glycosphingolipids	University of Massachusetts, Worcester	Metastatic melanoma	NCT00668512
IT CpG SD-101 TLR9 agonist and local radiotherapy and allogeneic HCT	Stanford University	Recurrent/progressive lymphoma after allogeneic HCT	NCT01745354
IT DCVax-Direct mature DC	Northwest Biotherapeutics	Locally advanced and metastatic solid tumors Liver cancer Colorectal cancer pancreatic cancer metastatic melanoma	NCT01882946
IT Transgenic oncolytic adenovirus expressing IL-12	Ziopharm	Metastatic melanoma	NCT01397708
IT Recombinant vesicular stomatitis virus expressing IFN-β	Mayo Clinic	Hepatocellular carcinoma	NCT01628640
IT Adenoviral vector delivery of the human IL-12 cDNA	Mount Sinai School of Medicine National Cancer Institute	Breast cancer Liver metastases secondary to colorectal cancer	NCT00849459 NCT00072098
IT INGN 241 Nonreplicating adenovector expressing IL-24	Introgen Therapeutics	Metastatic melanoma	NCT00116363
IT Injections of DCs and rituximab	Oslo University Hospital Norwegian Cancer Society Helse Sor-Ost	Follicular lymphoma	NCT01926639
IT AdGVEGR.TNF.11D Transgenic oncolytic adenovirus expressing TNF and local radiotherapy	GenVec NIH	Prostate cancer	NCT01048151
IT AdCD40L Transgenic oncolytic adenovirus expressing CD40L and low-dose cyclophosphamide	Uppsala University	Metastatic melanoma	NCT01455259
IT BCG and IV ipilimumab (anti–CTLA-4)	Ludwig Institute for Cancer Research BMS	Metastatic melanoma	NCT01838200
IT Bioengineered allogeneic immune cells (AlloStim) after cryoablation	Immunovative Therapies, Ltd.	Metastatic breast cancer	NCT01741038
IT Bioengineered allogeneic immune cells (AlloStim) after radiofrequency ablation	Immunovative Therapies, Ltd.	Refractory liver cancer	NCT01923233
IT IFN-β or local radiotherapy and IV MCPyV tumor age-specific polyclonal autologous $CD8^+$ T cells and SC rIL-2	Fred Hutchinson Cancer Research Center NIH	Merkel cell carcinoma	NCT01758458

microenvironment than do systemic infusions. Intratumoral delivery of immunostimulating agents should, therefore, provide lower toxicity of ISmAbs and better efficacy of PRRagos. However, this strategy has practical limitations. Only accessible sites of sufficient size can be injected. This could be an issue, especially if repeated injections are

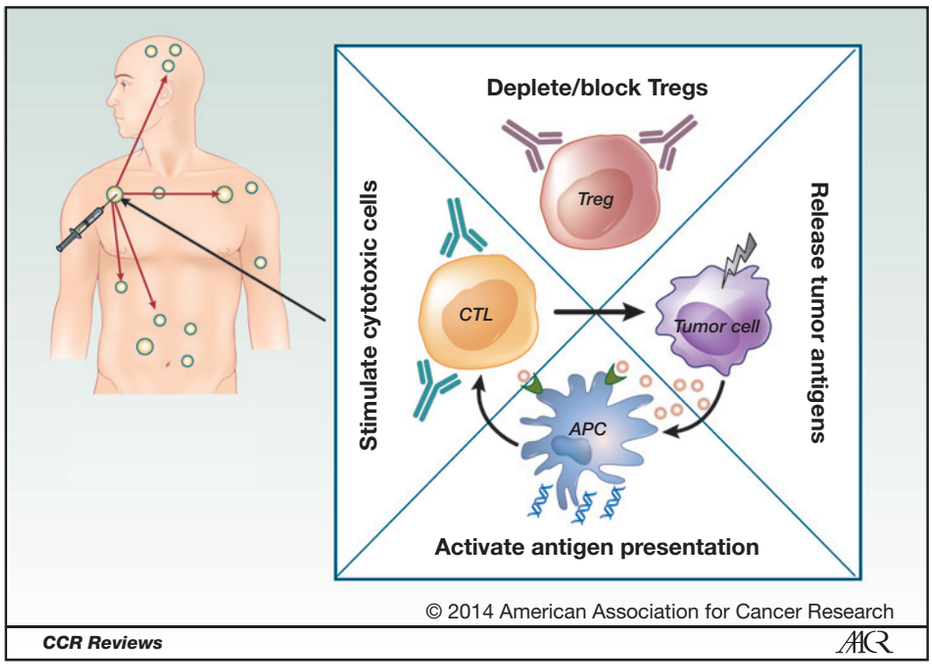

Figure 1. The ideal intratumoral combination. To trigger an efficient systemic antitumor immune response combination, four physiologic issues should be addressed with targeted therapies. First, tumor-specific Tregs should be depleted from the tumor microenvironment. This can be performed with ADCC-compatible isotypes of mAbs targeting costimulatory molecules expressed by Tregs upon recognition of tumor cognate antigens (e.g., IgG1 anti–CTLA-4 in humans). Second, tumor antigens should be released upon tumor cell death, and this should be performed with cytotoxic drugs generating immunogenic cell death, but sparing at least systemic white blood cells (e.g., local radiotherapy). Third, APCs should be activated with proinflammatory drugs (e.g., TLR-4 or TLR-9 agonists). Four, cytotoxic cells (NK and T cells) could be enhanced with agonistic, non-ADCC inducers, mAbs (e.g., IgG4 CD137 agonist).

needed to trigger the adaptive immune response. Beyond classical methods such as catheterization for continuous delivery or slow-release chemical complexes (e.g., PEGylated drugs), new modes of delivery could be eventually contemplated. For instance, antibody–drug conjugates or versatile nanomolecule platforms could be used for specific intratumoral homing of immunostimulating drugs. Devices allowing external activation of intratumoral drugs after systemic administration could also be tested (e.g., wavelength-specific drug photoactivation). Eventually, a better knowledge of the biology of cancers should allow identification of enzymes expressed in the tumor microenvironment that could specifically activate prodrugs locally that would have been delivered systemically.

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