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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]

[1] Centre de Recherche en Cancérologie de Lyon, UMR INSERM U1052 CNRS 5286, Centre Léon Bérard, Université de Lyon, Lyon, France.

[2] Department of Medicine, Division of Oncology, Stanford University, Stanford, California, USA.

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 anti-tumor immune response. Two major families of immunostimulatory drugs are currently in clinical development: pattern recognition receptor agonists (PRRagos) and immunostimulatory monoclonal antibodies (ISmAbs). Despite their immune targeted design, these agents have so far been developed clinically as if they were typical anti-cancer drugs. Here, we review the limitations of this conventional approach, specifically addressing the shortcomings of the usual schedules of intravenous infusions every two or three 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 pre-clinical and clinical evidence that a therapeutic systemic anti-tumor immune response can be generated upon intra-tumoral immunomodulation. Moreover, pre-clinical results have shown that therapeutic synergy can be obtained by combining PRRagos and ISmAbs to the local tumor site.

Keywords

Immunomodulation; Intralesional Injections; Cancer; Active Immunotherapy

Introduction

Major efforts have been made over the last 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 a 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

Corresponding author: Aurelien Marabelle (aurelien.marabelle@lyon.unicancer.fr) Léon Bérard Cancer Center, 28 rue Laennec, 69008 Lyon, France..

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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 in order to reverse the cancer-induced immunotolerance and allow an anti-tumor 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 NSCLC, diseases with low sensitivity to conventional cytotoxic therapies. The consequence of these positive results is a paradigm shift in oncology where 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 (PRRagos) and immunostimulatory mAbs (ISmAbs). Immune cells expressing the targets of these new drugs are present within the tumor micro-environment. Interestingly, evidence is accumulating to support the idea that these new drugs work by targeting intratumoral immune cells. Therefore, as opposed to conventional anti-cancer drugs, these immunostimulatory drugs can be delivered directly into the tumor, even at a single site, and generate a systemic anti-tumor immune response. This intratumoral delivery can trigger even more potent anti-tumor immune responses while causing less auto immune toxicity. Interestingly, in pre-clinical models only certain combinations of immunomodulatory agents are additive or synergistic in their therapeutic effects and induce curative systemic anti-tumor immunity. Here we will review the evidence for the effectiveness of intra-tumoral immunization.

Reversing tumor tolerance and boosting the anti-tumor immune response by targeting intratumoral PRRs

PRRs is a constantly growing family of receptors having the ability to recognize pathogen associated molecular patterns (PAMPs) such as bacterial cell wall molecules or viral DNA, and damage associated molecular patterns (DAMPs) released upon cell death, stress or tissue injury. Toll-Like Receptors (TLRs), a sub-family of PRRs, are highly expressed by immune cells from both myeloid and lymphoid lineages that infiltrate the tumor micro-environment, such as tumor-associated macrophages (TAMs), plasmacytoid and myeloid Dendritic cells (pDCs & mDCs), CD4+ and CD8+ T-cells, regulatory T-cells (Tregs), NK cells and B-cells (Table I). The pattern and level of expression of TLRs can vary depending on the immune cell lineages subsets (e.g mDCs subsets) and their state of activation (e.g upon BCR stimulation for B-cells) (2,3). The level of infiltration of some of these cells has a prognostic value in many cancer types (Table II).

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

Upon stimulation by their ligands, TLRs trigger the activation of the host cells (notably APCs) and the secretion of pro-inflammatory cytokines such as type I interferons (IFNs), 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 play a role in immune responses against tumor cells. Indeed, TLR stimulation of APCs within mice and human tumor micro-environment 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 anti-tumor immune response.

TLRs can also be expressed by tumor cells. The direct activation of TLRs on cancer cells can result into 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 called DAMPs which can stimulate the immune cells surrounding the tumor cells. This phenomenon has been well illustrated with HMGB1, an intra-cellular protein released in the tumor milieu upon 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 pro-oncogenic effect and stimulate the proliferation of cancer cells; see recent review in this journal (11).

Intra-tumoral immune stimulation can also be obtained by targeting intra-tumoral RIG-I like receptors (RLRs). 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 into its death by apoptosis (12). Such cytokine and TAAs release can also result in the activation of the anti-tumor 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 intra-tumoral delivery of oncolytic viruses.

Using tumors as their own vaccines: intra-tumoral delivery of PRRagos in human cancers

Tumor responses upon intra-tumoral delivery of pathogens have been described since the end of the XIXth century. Dr William Coley, a surgeon at what would become later the Memorial Sloan Kettering hospital in New York City, turned the phenomenon into a medical practice. He confirmed that intratumoral injections of extracts from bacteria responsible of erysipelas (Streptococcus pneumoniae and Serratia marcescens), could cure solid tumors (15). Later, accumulating pre-clinical evidence supported the use of BCG for cancer therapy (16). Clinicians reported the therapeutic benefits of intra-tumoral injections of BCG in several types of cancers such as melanoma (17–20) or squamous cell carcinoma of the head and neck (21). MD Anderson hospital reported up to 2500 patients with all types of cancer treated with BCG, including scarification of the tumors (22). Interestingly, Morton et al and Sparks et al reported that in patients with metastatic melanoma, intra-tumoral 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 et al reviewed 12 studies of intra-tumoral BCG in patients with metastatic cutaneous melanoma and found that injected tumors showed regression in 58% of the cases, and that distant, non-injected, tumor sites showed regression in 14% of the cases (23). Tokunaga et al identified that the therapeutic effects of BCG was partly due to the pro-inflammatory 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 PRRagos molecules seems to be as efficient as live bacteria injections to induce local control of tumors. Topical imiquimod has 70 – 90% clearance rates in superficial skin cancers such as Basal Cell Carcinomas and Squamous Cell carcinoma (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 intra-lesional IL-2 (28). Imiquimod in combination with intra-lesional BCG was able to induce complete remission in 5 out of 9 patients with cutaneous melanoma (29).

Intra-tumoral PRRagos can also generate some levels of systemic anti-tumor immunity inducing tumor responses in distant, uninjected, tumor sites. Repeated intra-tumoral CpG (PF-3512676) at one single tumor site together with a 2×2Gy 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 out of 15 patients with metastatic cutaneous T-cell lymphoma (7). The ability of intra-tumoral PRRagos to generate a systemic anti-tumor immune response has been also studied in pre-clinical models. In mice like in humans, intra-tumoral PRRagos usually triggers a local cytotoxic anti-tumor immune response which can result in complete regression of the injected tumor, but which has limited effect on the distant, uninjected tumor sites (8,30).

Mode of action of therapeutic intra-tumoral PRRagos

The local delivery of these immune stimulatory drugs is supported by the fact that many cells of the tumor micro-environment express PRRs (Table I). The mechanistic of intra-tumoral PRRagos therapeutic effect is multi-factorial, depending on the tumor cell type, the tumor micro-environment, 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 therefore helping the generation of an anti-tumor immune response (8,31). The cytokines released upon CpG injections have been shown to induce in an antigen non-specific 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 sub-cutaneous mouse melanoma tumors mediated by a direct killing of tumor cells by pDCs via a TRAIL/DR5 & Granzyme B mechanism and independently of adaptive immune cells (33). Shime et al 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 antigen presenting cells (B-cells, DCs, TAMs and other myeloid derived suppressor cells) mediated by pro-inflammatory cytokine secretion and upregulation of costimulatory molecules on their surface. Indeed, pre-clinical results have recently demonstrated in mice that intra-tumoral delivery of PRRagos stimulates the anti-tumor immune response via the activation of antigen presenting cells infiltrating the tumors (high expression of MHC II, CD80 and CD86) (6,8). This common feature is a prerequisite for mounting an efficient adaptive anti-tumor immune response against TAAs, but it does not address efficiently the issues of immunosuppressive tumor infiltrating Tregs, and anergic/exhausted tumor infiltrating or peri-tumoral cytotoxic T-cells (35).

Breaking the tumor tolerance and boosting the anti-tumor 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 the tumor immunotolerance and stimulate the anti-tumor immune response. Many of them are currently in clinical development (Table III) (36). Interestingly, these checkpoint molecules have been described to be highly expressed by immune cells infiltrating the tumor micro-environment (Table I).

The most clinically advanced of these new ISmAbs is the antagonistic anti-CTLA-4 ipilimumab (Yervoy*, BMS) which is FDA/EMA approved for the treatment of metastatic melanoma. In two subsequent randomized Phase III clinical trials, systemic intra-venous

therapy with ipilimumab generates long lasting tumor responses in up to 20% of patients with refractory/relapsing melanoma (37,38). However this therapy is associated with major auto-immune toxicities requiring high dose steroids in about 60% of the patients treated. Anti-CTLA-4 anti-tumor efficacy has been so far explained by the ability of this antagonistic mAb 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 intra-tumoral depletion of Tregs rather than an interaction with CD4+ effector T-cells (39). Indeed, intra-tumoral tumor-specific Tregs express high levels of CTLA-4 and are depleted upon therapy with anti-CTLA-4 via FcγR+ tumor infiltrating cells (40–42). These results can explain the systemic anti-tumor immune response that can be generated in mouse models with only local low dose delivery of anti-CTLA-4. Fransen et al demonstrated recently that low doses of anti-CTLA-4 delivered into a water-in-oil emulsion adjuvant (Montanide ISA 51) around an established mouse colon carcinoma tumor was able to eradicate the local tumor and prevent the development of tumors at a distant non injected site (43). Interestingly, this intra-tumoral Treg depletion also explains the in vivo efficacy of agonistic antibodies targeting the co-stimulatory molecules GITR and OX40 (40,42). These results open a new perspective on the mechanism of action of these ISmAbs and emphasize on the importance of their design, especially their isotype.

Systemic tumor responses upon intra-tumoral immunomodulation

In humans, rare observations of systemic tumor responses upon local irradiation have been reported historically and are referred as bystander effects or the “abscopal” effect (44). The incidence of this abscopal effect seems to be potentiated when local irradiation is combined to an immune modulatory strategy. As above mentioned, local irradiation combined to intra-tumoral 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 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 GM-CSF, IL-12 or type I IFN. Although not yet clearly defined, due to their pathogen structure all these viruses should also have PRRagos properties from their capside proteins or internal nucleic acids. For instance, DNA virus can be turned into dsRNA and subsequently activate RLRs (48). Interestingly, intra-tumoral delivery of such viruses is able to generate a systemic anti-tumor immune response. Intra-tumoral JX-594/TG6006 oncolytic virus in 14 patients with primary liver tumors or metastatic intra-hepatic 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 HCC were the same disease control was obtained in injected and distant sites (50). Many intra-tumoral immunization clinical trials are currently ongoing, using intra-tumoral immunostimulatory products with the aim of generating a systemic anti-tumor immune response (Table IV).

Pre-clinical models have recently demonstrated that immunostimulatory drug's efficacy is potentiated upon intra-tumoral injections. The hypothesis behind such practice is that by delivering locally high concentrations of immunomodulatory drug, we could trigger a more efficient anti-tumor immune response. Dubrot et al showed that intra-tumoral injections of type I IFN alone or anti-CD137 systemic therapy alone have little therapeutic effect against the MC38 mouse colon carcinoma (51). However, the combinations of intra-tumoral IFNα together with systemic high dose anti-CD137 synergize and generate immune mediated

tumor responses at distant non injected sites. Subsequently, the same team showed in the same colon carcinoma model that intra-tumoral low doses of anti-CD137 (5ug i.p. instead of 100ug i.p./injection) injected into one tumor site was sufficient to eradicate both injected and distant non injected 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 intra-tumoral anti-CD137 + systemic anti-PD-L1 was able to cure most of the mice. Most importantly, intra-tumoral injections of low doses anti-CD137 avoided auto-immune hepatocytolysis and liver T-cell infiltration that is generated by the same drug when administered systemically. Like for anti-OX40 and anti-GITR, local anti-CD137 effect could also be mediated via intra-tumoral Treg depletion because Tregs also express high levels of CD137. Intra-tumoral 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 anti-tumor immune response was weak as it only slowed the tumor growth of distant sites. Besides the difference of tumor model (B16 melanoma instead of MC38), this anti-CD137 + IL2 strategy might be not optimal at generating a potent systemic anti-tumor immune response due to the stimulatory properties of IL-2 on Tregs (54).

Fransen et al showed that for the same anti-tumor efficacy, liver enzymes were lower upon local low doses anti-CTLA-4 rather than for systemic high dose (43). Simmons et al 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 a systemic anti-tumor immunity while preventing the rise of circulating levels of auto-immunity markers (ANA, ssDNA and dsDNA) happening upon prolonged anti-CTLA-4 therapy (55). The lower toxicity of local low dose immunomodulation vs 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 intra-tumoral combinations. A triple combination of intra-tumoral 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 anti-tumor immune response able to eradicate distant metastatic tumor sites, including in the central nervous system in almost all mice treated. This local combination strategy generated a better CD8+ memory anti-tumor 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 intra-tumoral Tregs. The requirement of having CpG co-injected into the tumor can be explained by recent results showing that the in vivo therapeutic effects of ISmAbs via Treg depletion relies probably on ADCC (41,42). Because CpG stimulates ADCC, it might explain why it potentiates Treg depletion upon combination with ISmAbs (56). Together this data suggest that to generate an efficient systemic adaptive anti-tumor immune response, intra-tumoral immunization strategies should combine a Treg depletion to an immunogenic tumor cell death and an activation of APC's (Figure 1).

Practical and clinical consequences of local delivery of immunostimulatory drugs

Local delivery of immune stimulating 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 micro-environment than do systemic infusions. Intra-tumoral delivery of immune stimulating agents should therefore provide a 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 needed to trigger the adaptive immune response. Beyond classical methods such as catheter for continuous delivery or slow release chemical complexes (e.g PEG-ylated drugs), new ways of delivering them could be eventually contemplated. For instance, antibody-drug conjugates or versatile nano-molecule platforms could be used for specific intra-tumoral homing of immune stimulating drugs. Devices allowing external activation of intra-tumoral drugs after systemic administration could also be tested (e.g. wave-length specific drug photoactivation). Eventually, a better knowledge of the biology of cancers should allow to identify enzymes expressed in the tumor micro-environment which could specifically activate pro-drugs locally that would have been delivered systemically.

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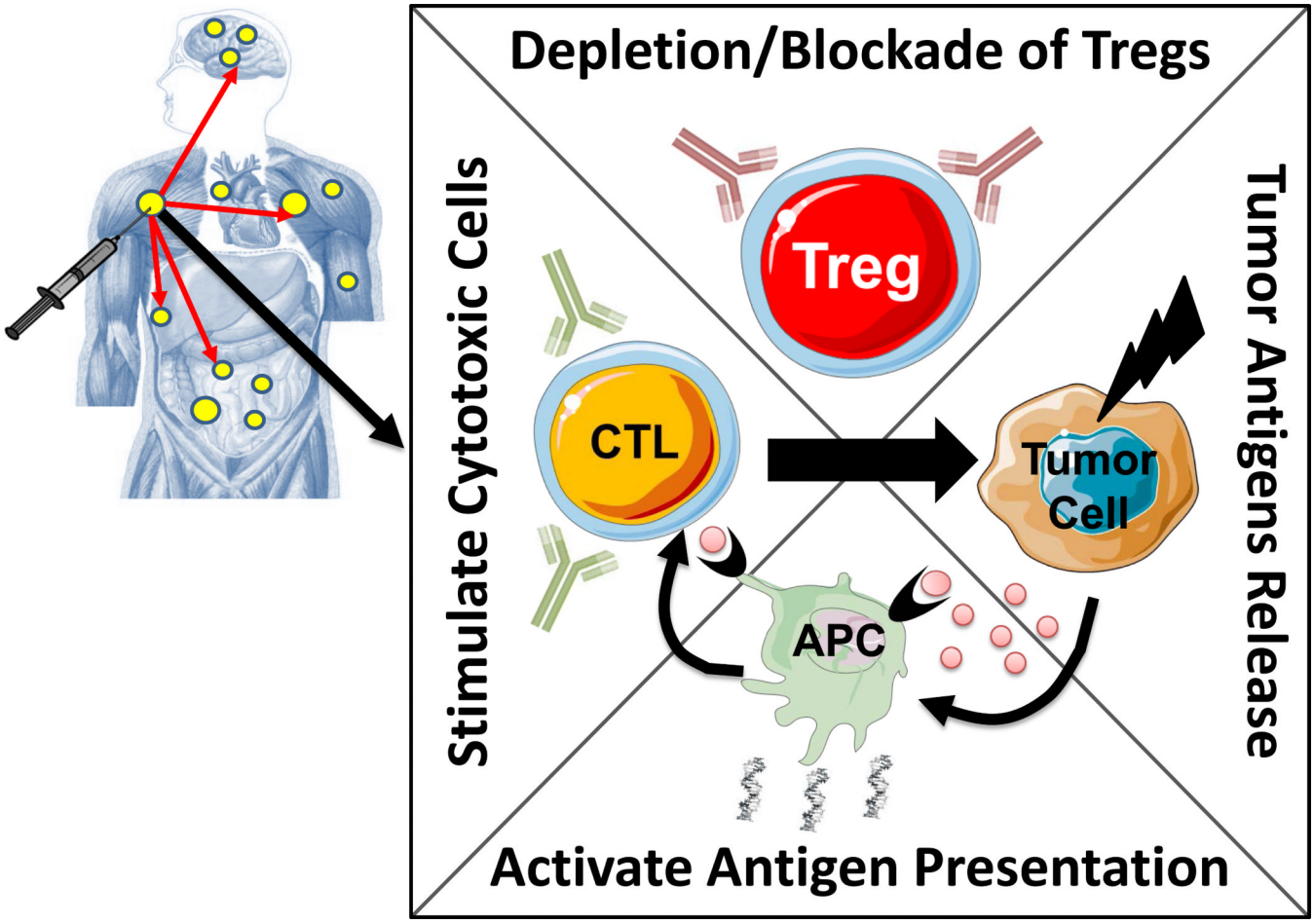

Figure 1. The ideal intra-tumoral combination

In order to trigger an efficient systemic anti-tumor immune response combination, four physiological issues should be addressed with targeted therapies. First, tumor-specific regulatory T-cells (Tregs) should be depleted from the tumor micro-environment. This can be performed with ADCC-compatible isotypes of monoclonal antibodies (mAbs) targeting costimulatory molecules expressed by T-regs 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, antigen-presenting cells should be activated with pro-inflammatory drugs (e.g: TLR-4 or TLR-9 agonists). Four, cytotoxic cells (NK, T-cells) could be enhanced with agonistic, non-ADCC inducers, mAbs (e.g: IgG4 CD137 agonist).

Table I

Immunostimulatory targets on tumor-infiltrating human immune cells.

Cell Type	PRRagos Targets	ISmAbs 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

TLR: Toll-like Receptor; KIR: killer immunoglobulin-like receptors; PD-1: Programmed Cell Death 1; PD-L1: PD-1 ligand; OX40: also known as CD134; CD137 also known as 4-1BB.

Table II

Diversity of Cancer Types with Prognostic Immune Contexture.

Tumor-Infiltrating Immune Cell	Pronostic Value in	Ref
Dendritic Cells (DCs)	Ovarian Cancer	(57)
	Breast Cancer	(58)
	Colon Cancer	(59)
	Lung	(60)
	Oral Squamous Cell Carcinoma	(61)
	Melanoma	(62)
	Gastric cancer	(63)
	Gallbladder Carcinoma	(64)
Tumor Associated Macrophages (TAMs)	Neuroblastoma	(65)
	Osteosarcoma	(66)
	Breast Cancer	(67)
	Ewing Sarcoma	(68)
Regulatory T-cells (Tregs)	NSCLC	(69)
	Pancreatic	(70)
	Gastric	(71)
	Hepatocellular Carcinoma	(72)
	Ovarian Carcinoma	(73)
CD8+ T-cells	Colon cancer	(74)
	NSCLC	(75)
	Ovarian	(76)
	Melanoma	(77)

Tumor infiltration by DCs, TAMs and Tregs are 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 to determine the activation status / the antigen specificity of these immune cells and their proportion in precise areas within the tumor micro-environment.

Table III

Immuno-stimulatory mAbs currently in Clinical Development.

Therapeutic Molecule	Drug Currently in Development		
	Name	Sponsor	OngoingTrials
Anti-CD137 (4-1BB)	PF-05082566	Pfizer	NCT01307267
	Urelumab (BMS-663513)	BMS	NCT01471210
			NCT01775631
Anti-CD134 (OX40)	Anti-OX40 antibody	Providence Health & Services	NCT01642290
			NCT01862900
			NCT01303705
Anti-PD-1	Nivolumab (MDX 1106/BMS-936558/ONO4538)	BMS	NCT01658878
			NCT01629758
			NCT01176461
			NCT01968109
			NCT01714739
			NCT01592370
			NCT01673867
			NCT01721746
			NCT01721772
			NCT01668784
			NCT01844505
			NCT01642004
	Pidilizumab (CT-011)	Curetech	NCT01441765
			NCT01096602
			NCT01067287
			NCT01952769
			NCT01313416
	MK-3475/SCH900475	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

Therapeutic Molecule	Drug Currently in Development		
	Name	Sponsor	OngoingTrials
			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

Table IV

Ongoing Intra-Tumoral Immunization Trials.

Trial Design	Trial Sponsor	Disease	Trial #
IT ipilimumab (anti-CTLA-4) & Local Radiotherapy	Stanford University	B,T & NK-cell lymphomas Colon & Rectal cancers	NCT01769222
IT ipilimumab (anti-CTLA-4) & IT IL-2	University of Utah	Metastatic Melanoma	NCT01672450
IT IL-2 & IV ipilimumab (anti-CTLA-4)	University Hospital Tuebingen	Metastatic Melanoma	NCT01480323
IT Talimogene laherparepvec Transgenic Oncolytic Virus expressing GM-CSF & IV ipilimumab (anti-CTLA-4)	Amgen	Metastatic Melanoma	NCT01740297
IT Poly-ICLC TLR3 agonist & IT Flt3L cytokine & 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 & Local Radiotherapy & Allogeneic HCT	Stanford University	Recurrent/Progressive Lymphoma After Allogeneic HCT	NCT01745354
IT DCVax-Direct Mature DC	Northwest Biotherapeutics	Locally Advanced & 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-beta	Mayo Clinic	Hepatocellular Carcinoma	NCT01628640
IT Adenoviral Vector Delivery of the Human IL-12 cDNA	Mount Sinai School of Medicine National Cancer Institute (NCI)	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 Dendritic Cells and Rituximab	Oslo University Hospital Norwegian Cancer Society Helse Sor-Ost	Follicular Lymphoma	NCT01926639
IT AdGVEGR.TNF.11D Transgenic Oncolytic Adenovirus expressing TNF & Local Radiotherapy	GenVec National Institutes of Health (NIH)	Prostate Cancer	NCT01048151
IT AdCD40L Transgenic Oncolytic Adenovirus expressing CD40L & Low dose cyclophosphamide	Uppsala University	Metastatic Melanoma	NCT01455259
IT BCG & 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- beta or Local Radiotherapy & IV MCPyV Tumor Ag-specific polyclonal autologous CD8+ T cells & SC rIL-2	Fred Hutchinson Cancer Research Center NIH	Merkel Cell Carcinoma	NCT01758458

IL-2: interleukin-2. NCI : National Cancer Institute. NIH : National Institutes of Health.