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Annals of Oncology 28 (Supplement 12): xii33–xii43, 2017
doi:10.1093/annonc/mdx683

REVIEW

Intratumoral immunotherapy: using the tumor as the remedy

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Immune checkpoint-targeted monoclonal antibodies directed at Programmed Death Receptor 1 (PD-1), Programmed Death Ligand 1 (PD-L1) and Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4) are currently revolutionizing the prognosis of many cancers. By blocking co-inhibitory receptors expressed by antitumor T cells, these antibodies can break the immune tolerance against tumor cells and allow the generation of durable cancer immunity. Benefits in overall survival over conventional therapies have been demonstrated for patients treated with these immunotherapies, leading to multiple approvals of such therapies by regulatory authorities. However, only a minority of patients develop an objective tumor response with long-term survival benefits. Moreover, the systemic delivery of immunotherapies can be responsible for severe auto-immune toxicities. This risk increases dramatically with anti-PD(L)1 and anti-CTLA-4 combinations and currently hampers the development of triple combination immunotherapies. In addition, the price of these novel treatments is probably too high to be reimbursed by health insurances for all the potential indications where immunotherapy has shown activity (i.e. in more than 30 different cancer types). Intratumoral immunotherapy is a therapeutic strategy which aims to use the tumor as its own vaccine. Upon direct injections into the tumor, a high concentration of immunostimulatory products can be achieved *in situ*, while using small amounts of drugs. Local delivery of immunotherapies allows multiple combination therapies, while preventing significant systemic exposure and off-target toxicities. Despite being uncertain of the dominant epitopes of a given cancer, one can therefore trigger an immune response against the relevant neo-antigens or tumor-associated antigens without the need for their characterization. Such immune stimulation can induce a strong priming of the cancer immunity locally while generating systemic (abscopal) tumor responses, thanks to the circulation of properly activated antitumor immune cells. While addressing many of the current limitations of cancer immunotherapy development, intratumoral immunotherapy also offers a unique opportunity to better understand the dynamics of cancer immunity by allowing sequential and multifocal biopsies at every tumor injection.

Key words: intratumoral, cancer, immunotherapy, *in situ* immunization, *in situ* vaccine

Introduction

The history and first clinical successes of cancer immunotherapy started with intratumoral immunotherapy. At the end of the 19th century, Dr William Coley, a surgeon from New York city, started to prospectively treat cancer patients with intratumoral injections of live bacteria and subsequently with bacterial 'toxins' [1]. He treated hundreds of patients and reported multiple durable tumor responses [2]. However, his supervisor, Professor James Ewing, chose another novel cancer treatment strategy, radiotherapy, which offered more safety (since no antibiotics were available at that time), better reproducibility, and more practicality than intratumoral therapy. Although they both co-founded the American Association of Cancer Research, Ewing became renowned and Coley was forgotten for a long time by the oncology community. Nonetheless, one of the first 'conventional' immunotherapy was developed from his past experience with the use of repeated transurethral instillations of Bacille de Calmette & Guérin (BCG) for the treatment of bladder cancers [3].

Immunotherapy is now again at the forefront of cancer research thanks to the broad clinical successes of immune check

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point-targeted monoclonal antibodies (ICT mAbs). The spectrum of activity of anti-Programmed Death Receptor 1 (PD-1)/Programmed Death Ligand 1 (PD-L1) mAbs across more than 30 different cancer types, and the overall survival benefits that they provide resulting from durable disease control have recently demonstrated the validity of targeting the immune system to treat cancers. However, ICT mAbs harbor some major limitations: (i) their long-term benefits are restricted to a minority of patients (i.e. objective responders), (ii) their toxicity (especially when used as combinations) may limit their use because severe irAEs can be irreversible and sometimes life threatening and (iii) their cost might not be sustainable for healthcare providers if all cancers become, at some point, eligible for ICT mAbs (so-called financial toxicity). Intratumoral immunotherapy can address these issues by (i) providing a better priming of the antitumor immune response, (ii) avoiding off-target toxicities and (iii) requiring a lower amount of medications per patient.

In theory, an efficient T-cell-mediated adaptive antitumor immune response requires two phases: the priming phase (generation of antitumor T cells) and the effector phase (destruction of the cancer cells by the T cells). Anti-PD-1/PDL-1 mAbs are thought to work during the effector phase and thus might require a pre-existing antitumor immunity in patients. Conversely, patients with no pre-existing antitumor immunity, and/or no intra-tumoral T-cell infiltrates or PD-L1 expression (so-called cold/uninflamed or immune-excluded tumors) are usually considered to be resistant to ICT mAbs.

In this review, we will demonstrate how intratumoral immunotherapies, by priming antitumor T cells and/or allowing their intratumoral homing/function, may offer new combinatorial opportunities to turn 'cold' tumors into 'hot' tumors and overcome resistance to PD-1/PD-L1/CTLA-4 immune check point-targeted immunotherapies.

Principles of intratumoral immunotherapy

In situ priming of antitumor immunity

Intratumoral immunotherapy aims at generating a potent priming of antitumor immunity for a systemic and durable clinical benefit (Figure 1). It could either prime an immune response against a tumor that has no pre-existing antitumor immunity (e.g. with intratumoral cytokines or bispecific T-cell engaging mAbs) or prime/boost tumors that are insufficiently immunogenic [e.g. boosting of tumor infiltrating T cells or tertiary lymphoid structures with agonistic immune check points or toll-like receptor (TLR) agonists]. Direct intratumoral delivery allows high local concentrations of a drug to be achieved and increases the bioavailability of immunostimulatory products. Therefore, intratumoral immunotherapy is, in theory, of interest for any immunostimulatory drug which has a dose–effect relationship (TLR agonists, agonistic mAbs, anti-CTLA-4, etc.) and dose-limiting toxicities. Also, intratumoral immunotherapy can allow for the use of multiple combinations of immunostimulatory drugs that are too toxic when used systemically. Therefore, intratumoral immunotherapy can be used as a versatile platform for modulating key components of the antitumor immune response, such as concomitant targeting of T cells, B cells and macrophages.

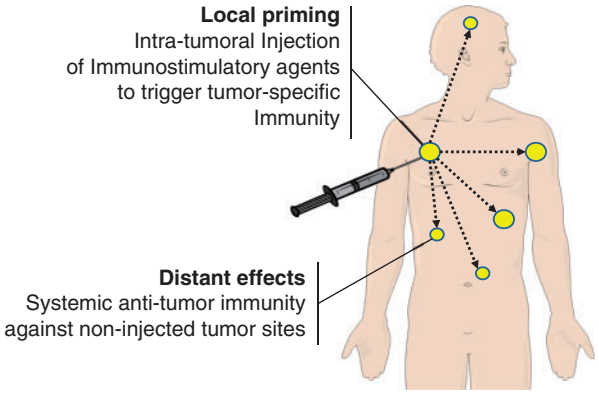

Figure 1. Principle of intratumoral immunotherapy. Intratumoral injection of immunostimulatory agents for efficient priming of the antitumor immune response. Upon circulation into the lymphatics and blood vessels, effectors of the antitumor immune response can attack the noninjected, distant, tumor lesions. The ability of injecting several tumor lesions over time can enable a prime-boosting effect on the repertoire of the polyclonal adaptive antitumor immune response, which could better address the heterogeneity of cancer cell subclones across lesions.

Use the tumor as its own vaccine (to better address the heterogeneity of cancers)

Intratumoral immunotherapy aims to use the tumor as its own vaccine by generating antitumor immunity against cancer cell antigens. It allows being agnostic from the nature of the most immunogenic cancer cell antigens [neo-antigens, tumor-associated carcino-embryonic antigens, glycopeptides, major histocompatibility complex (MHC) I or II restricted] while enabling the generation of a polyclonal antitumor immune response against multiple cancer targets which provides a better opportunity to address the heterogeneity of the disease [4]. The heterogeneity of a cancer is due to the accumulation of mutations in the cancer cell genome over time. Every cancer daughter cell can acquire mutations that are not present in the parent cancer cell. These mutations accumulate over time and the mutation profile can be different within areas of the same tumor lesion and across tumor lesions [5–7]. Most interestingly, recent data has shown that there is also an intratumor heterogeneity of the T-cell infiltrates and of their TCR repertoire which might be related to the local level of somatic DNA mutations [8]. Intratumoral immunotherapy would therefore be of interest for its ability to generate an antitumor immune response against all the cancer cell subclones present in a tumor lesion. The ability to inject *in situ* multiple tumor lesions in the same patient would enhance the chances of generating a polyclonal immune response across antigens shared by all the cancer cells. The ability to change over time the tumor lesions that are injected could lead to a beneficial prime-boosting effect on the antitumor immune response [9]. Also, the opportunity to generate both T-cell and B-cell antitumor immune responses upon intratumoral immunotherapy combinations could circumvent some

of the escape mechanisms seen with ICT mAbs monotherapies [e.g. loss of human leukocyte antigen (HLA)-I expression on cancer cells].

Avoid systemic toxicities

Upon intratumoral injections of immunostimulatory products, high concentrations and bioavailability of drugs can be reached locally while the total dose per body weight and the actual systemic exposure to the products can remain (very) low. Although little is known about the pathophysiology of irAEs that are generated by cancer immunotherapies, it is probable that most, if not all, of these toxicities are due to off-target effects. Indeed, the systemic delivery of immunostimulatory products exposes healthy tissues to drugs which can either break the self-immune tolerance (e.g. tissue-specific autoimmunity) or activate in an inappropriate manner the whole immune system (e.g. hemophagocytic and cytokine release syndromes). For instance, the recent randomized phase III trials of ipilimumab at 3 versus 10 mg/kg has indeed demonstrated that the higher the dose of systemic anti-CTLA-4, the higher the toxicity [10, 11]. The local delivery of a high concentration of immunostimulatory drugs can be carried out while working with much lower doses than the usual systemic doses. Therefore, intratumoral immunotherapy should lead to a much lower systemic diffusion and healthy tissues exposure of the immunostimulatory drugs and diminish the incidence of irAEs.

Key clinical results with intratumoral immunotherapy

Bacillus Calmette–Guérin (BCG)

During the 1970s, intratumoral BCG was tested in melanoma and head and neck squamous cell carcinoma. Both local and distant activity were reported with local response rates at around 50% and distant (noninjected lesions) response rates at around 15% [12]. The clinical activity of BCG has led to its use for the treatment of superficial bladder cancers [3, 13]. However, the current worldwide shortage of BCG production hampers its development in the clinic [14]. Over the last 20 years, several pre-clinical reports have identified the immune stimulatory parts that are present in that bacteria [15, 16]. This rationale supports the current development of synthetic agonists of pattern recognition receptors that are more druggable than the production of Generic good manufacturing practice (GMP) pathogens [17].

Intratumoral TLRs

Topical TLR agonists have been widely used for the treatment of cutaneous cancer lesions. Imiquimod, a TLR-7/8 agonist, has shown potent antitumor activity and is now approved for the treatment of superficial basal cell carcinomas, actinic keratosis, and genital warts [18, 19]. Topical imiquimod has been shown to increase the level of CD4 and CD8 T cells in the skin and the level of CD4 T cells in the sentinel lymph node of resected melanomas [20]. A phase I/II trial has tested the value of topical imiquimod in combination with intra-lesional interleukin (IL)-2 in 13 patients with cutaneous melanoma metastases. In this study, 182 tumor lesions were treated (137 purely cutaneous lesions and 41 subcutaneous nodules). Tumor responses were seen in 50.5% of the treated lesions (92/182) with 40.7% (74/182) of these being in complete response (91% of CRs being in the cutaneous lesions). Interestingly, patients with cutaneous disease experienced a marked slowing of the appearance of new cutaneous lesions and no cutaneous lesions, which previously responded reappeared on cessation of treatment [21]. Similarly, Kidner et al. [22] reported their experience of intralesional BCG in combination with topical imiquimod in melanoma patients. Five out of nine patients (56%) had complete regression of their in-transit metastases and one patient had a partial response. The mean interval between the first treatment and the complete resolution of in-transit metastases was 6.5 months (range, 2–12 months). With a mean follow-up of 35 months (range, 12–58 months), seven patients (78%) did not developed recurrent in-transit disease. Resiquimod, another TLR-7/8 agonist, has been shown to have significant activity in cutaneous T-cell lymphomas (CTCL). Rook et al. [23] have reported the results of a phase I trial of 12 patients with stage IA–IIA CTCL treated with topical resiquimod. Treated lesions significantly improved in 75% of patients and 30% had clearing of all treated lesions. Resiquimod also induced abscopal regression of untreated lesions. Two patients experienced complete clearing of their disease. T-cell receptor sequencing and flow cytometry studies of T cells from treated lesions showed a decrease in clonal malignant T cells in 90% of the patients and a complete eradication of malignant T cells in 30%. In all these reports of topical TLR agonists, adverse events were minor and largely limited to the skin. Intratumoral TLR agonists have also been tested in combination with mild (2×2 Gy) local irradiation in patients with B- and T-cell lymphomas. Brody et al. [24] have reported an objective response rate of 27% ($n = 4/15$ patients) in noninjected (abscopal) target lesions of patients with metastatic low grade B-cell lymphomas. Interestingly, an additional eight patients showed durable stable disease, including two patients with subclinical systemic tumor regressions. A greater magnitude of clinical response was correlated with fewer prior lines of therapies and with treatment-induced flu-like symptoms. Kim et al. [25] reported an objective response rate of 36% ($n = 5/14$ patients) with the same combination therapy in noninjected (abscopal) target lesions in patients with mycosis fungoides. In the biopsies carried out at injected sites, they found a significant decrease in CD25+/FoxP3+ T cells and antigen-presenting cells, and increased CD123+ pDCs upon intratumoral immunization. No antitumor humoral (IgG) response could be identified upon treatment.

Local irradiation

Interestingly, the local tissue damage and inflammation induced by radiotherapy has the opportunity to generate tumor antigen and release danger-associated molecular patterns [26]. Like intratumoral immunostimulatory drugs, local irradiation induces some systemic immune changes (e.g. systemic cytokine or chemokine increases) which can be useful for specific immunotherapy combinations [27, 28]. It has also been shown in preclinical models that irradiation efficacy partly relies on the immune system (notably type I interferons) and can generate antitumor immunity through immunogenic cell death, antigen release, MHC-I upregulation, and T-cell responses [29–32]. Therefore,

Table 1. Select clinical trials with intratumoral immunotherapy

Intratumoral agent	Type	Combo	Tumor/patient types	Phase	Response rate		Objective response rate (# of patients)	Comments	References
					Injected tumors (# of lesions)	Noninjected tumors (# of lesions)			
Oncolytic virus	T-VEC (GM-CSF encoding HSV-1)	–	Stage IIIB to IV melanoma (45% of stage IV M1b/c)	III Rando versus GM-CSF	64% ($n = 2116$)	• Uninjected visceral: 15% ($n = 177$) • Uninjected non visceral: 34% ($n = 981$)	26% ($n = 295$) in the T-VEC arm per modified WHO criteria	ORR, DRR and OS better in first line and in IIIB/C & M1a but no difference in M1b/c	[44, 45]
		αCTLA-4 Ipilimumab	Stage IIIB to IV Melanoma (49% of stage IV-M1b/c)	II Rando versus ipi alone	56% ($n = 89$)	Uninjected visceral: T-VEC+Ipi = 35% ($n = 31$); Ipi = 14% ($n = 22$) Uninjected nonvisceral: T-VEC + Ipi = 35% ($n = 37$); Ipi = 38% ($n = 80$)	T-VEC + ipi = 39% ($n = 98$) Ipi = 18% ($n = 100$) Per irRC criteria	ORR in M1b/c: T-VEC + ipi = 33% ($n = 48$); Ipi = 16% ($n = 43$)	JA Chesney, ASCO 2017. Abstract # 9509
		αPD-1 Pembrolizumab	Stage IIIB to IV melanoma (52% of stage IV-M1b/c	Ib	80% ($n = 50$)	Uninjected visceral: 28% ($n = 29$) Uninjected nonvisceral: 45% ($n = 20$)	57% (n=21) Per irRC criteria	CR in injected lesions: 70% ($n = 50$)	GV Long, ASCO 2016 Abstract # 9568
	CAVATAK (Coxsackie A)	αCTLA-4 Ipilimumab	Stage IIIC to IV melanoma (64% of stage IV –M1b/c)	Ib	N/D	Uninjected visceral: 47% ($n = 19$) Uninjected nonvisceral: 21% ($n = 29$)	50% ($n = 18$) Per irRC criteria	Responses in 37% ($n = 8$) anti-PD-1 refractory patients	B Curti SITC 2016 Abstract #140
		αPD-1 Pembrolizumab	Stage IIIB to IV melanoma 7/12 (58%) of stage IV-M1b/c	Ib	71% ($n = 17$)	86% ($n = 7$)	70% (n=10) Per irRC criteria		HL Kaufman, SITC 2016Abstract #328
Cytokine	IL-2	–	Stage IIIB to IV melanoma 7/48 (15%)	2	79% ($n = 894$)	N/D	N/D	CR+PR in stage III lesions: 97% ($n = 509$)Stage IV lesions: 56% (n = 385)	[49]
		αCTLA-4 Ipilimumab	Stage IIIB to IV melanoma 3/12 (25%) of stage IV-M1b/c	I	58% ($n = 12$)	N/D	30% ($n = 10$) Per irRC criteria		[51]
		Topical TLR7/8 Imiquimod	Stage III-IV melanoma	I/II	51% ($n = 182$)	N/D	N/D		[28]

Continued

Table 1. *Continued*

Intratumoral agent	Type	Combo	Tumor/patient types	Phase	Response rate		Objective response rate (# of patients)	Comments	References
					Injected tumors (# of lesions)	Noninjected tumors (# of lesions)			
Toll-Like receptor 9 agonist	PF-3512676	RT	Low grade B-cell lymphoma	I/II	87% ($n = 15$)	27% ($n = 15$)	27% ($n = 15$) Per IWG criteria		[24]
	PF-3512676	RT	T-cell lymphoma (mycosis fungoides)	I/II	N/D	N/D	36% ($n = 14$)		[25]
	SD-101	αPD-1 Pembrolizumab	Stage IIIC to IV melanoma [19/22 (86%) stage IV]	1b/2	N/D	N/D	41% ($n = 22$) Per RECIST criteria	Previously treated with: Apd1: 59% ($n = 22$); Actla4: 72% ($n = 22$)	ACF Leung, ASCO 2017 Abstract # 9550

CR, complete response; DRR, durable response rate (objective response lasting continuously>6 months); ORR, objective response rate; OS, overall survival; PR, partial response; Rando, Randomization; R, randomized; RT, radiotherapy.

irradiation has features of intratumoral immunotherapy. However, radiotherapy does not address the pre-existing immune tolerance against tumor antigens, and after an initial tumor tissue damage, negative feedback loops (e.g. proliferation of regulatory T cells, upregulation of CD39) will bring back tolerance and protect cancer cells from cytotoxic T cells [33]. In line with this rationale, preclinical data has shown that irradiation can overcome the resistance to anti-PD-L1 therapy in immunocompetent mouse models [34–36]. Most interestingly, abscopal responses have been reported in melanoma, NSCLC and Hodgkin lymphoma patients who were refractory to anti-CTLA-4 or anti-PD-1 therapy and received local palliative radiation therapy [37–42].

Oncolytic viruses

Oncolytic viruses offer an opportunity to provide intra-tumoral danger signals for the immune system (oncolytic viruses are pathogens) and an immunogenic cell death of cancer cells (oncolytic viruses can infect and kill cancer cells) [43]. They have demonstrated clinical activity in humans with melanoma, multiple myeloma and hepatocarcinoma [44–47]. Intratumoral oncolytic viruses have been shown to overcome resistance to anti-CTLA-4 therapy in preclinical models [48]. In humans, the combination of T-VEC, an HSV-1 GM-CSF encoding oncolytic virus, with ipilimumab has shown objective responses in 50% (9/18) of stage IIIb-M1c melanoma, significantly above the usual 10%–15% objective responses seen with anti-CTLA-4 agents (Table 1).

Intratumoral IL-2

Intralesional IL-2 cytokine therapy has been widely used for in-transit metastases of melanoma [49]. It has strong activity on injected lesions and can also generate abscopal responses (Table 1). However, as for oncolytic viruses, the activity of intralesional IL-2 is mostly active in stage III melanomas and in tumors that are small in size [50]. More recently, a combination of intralesional IL-2 with anti-CTLA-4 from a small phase I trial has been reported, showing local responses in 67% of patients ($n = 8/12$), abscopal responses in 89% of patients and an objective response rate by irRC of 40% [51].

More clinical results are expected in the near future taking into account the numerous intratumoral immunotherapy combinatorial approaches that are currently being tested in clinical trials (recently reviewed in [52] and [17]).

Advantages of intratumoral immunotherapy

As described in Table 2, intratumoral immunotherapy provides several advantages over conventional cancer immunotherapy strategies. As mentioned above, beyond their local priming effects, intratumoral immunotherapies have the opportunity to induce systemic changes (e.g. systemic cytokine releases, fever). Altogether, intratumoral immunotherapy offers an opportunity to design triple or quadruple combination therapies which could overcome the resistance to anti-PD-1/PD-L1 monotherapies and turn a majority of patients into long term responders.

doi:10.1093/annonc/mdx683 | xii37

Table 2. Advantages of intratumoral immunotherapy

Production	• Does not require antigen (Ag) identification nor isolation • Does not require *ex vivo* manipulation ('off the shelf') • Cheaper than other approaches, such as systemic administration (lower doses) or *ex situ* vaccines (no *ex vivo* manipulation)
Efficacy	• Direct/better bioavailability of immunostimulatory drugs (low doses may be efficient, high concentration within the tumor easier to reach) • May allow multiple combinations (due to reduced systemic toxicity) • Applicable to all patients (no Ag nor HLA-restriction) • Ag diversity: induces an antitumor immune response against the entire antigenic repertoire of a tumor (not limited to a few tumor associated antigens) • Antitumor immune response adapted to the patient's own tumor (highly personalized vaccine) • Local efficacy (localized disease, neo-adjuvant treatment) • Systemic efficacy (abscopal effect)
Safety	• Limited 'systemic' side-effects (may allow multiple combinations)
Immune monitoring	• Repeated injections allow for intratumoral immune monitoring

HLA, human leukocyte antigen.

By using conventional drugs, intratumoral immunotherapy offers an opportunity to build universal immunotherapy strategies rather than individualized immunotherapy strategies (e.g. neo-epitope vaccines). Also, repeating the intratumoral immunotherapy allows for sequential biopsies which can be used to build pre-emptive immunotherapy strategies. Such an approach would allow patients to be stratified and to build more personalized treatments based on the mechanisms of escape to immunotherapy.

Advantages over systemic delivery of immunostimulatory drugs

The bioavailability at the tumor site of many immunostimulatory drugs remains uncertain/suboptimal upon systemic delivery. For instance, we know that there is a positive correlation between the dose and efficacy of anti-CTLA-4 therapy in melanoma and that inter-individual variability impacts the systemic exposure to such drugs [10, 53]. We also know that a higher dose of anti-CTLA-4 generates a higher level of severe CTCAE grade 3–4 irAEs (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_40) [10, 54, 55]. Intratumoral anti-CTLA-4 would offer the opportunity to increase the bioavailability of the antibody into the tumor while avoiding its systemic toxicities. Moreover, combinations of anti-CTLA-4 with anti-PD-1/PD-L1 generate a much higher level of CTCAE grade 3–4 irAEs as opposed to anti-PD-1/PD-L1 or anti-CTLA-4 monotherapies [56, 57]. Some immunostimulatory drugs cannot be given systematically because of their systemic toxicity although they have shown potent immunostimulatory activities (e.g. agonistic anti-CD28 or anti-CD137 mAbs) [58, 59]. Other systemic immunotherapies are poorly tolerated when administered systemically (e.g. IL-2, interferons). Having the opportunity to test these compounds locally at much lower systemic doses could therefore take advantage of the dose-effect correlation of such products while avoiding their systemic toxicity.

Advantages over *ex situ* cancer vaccines

Ex situ vaccines (i.e. vaccines generated *ex vivo*), such as dendritic cell (DC) vaccines, have major limitations in comparison to *in situ* immunization approaches (Table 3). Such cell-based therapeutic strategies require logistics and GMP facilities that are both complex and expensive. Moreover, they need to be pulsed with tumor antigens (Ags) which need to be preidentified, isolated, GMP produced, and which are usually limited in number to a few Ags. Loading DC vaccines with tumor lysates adds another level of complexity level to the GMP process.

Neo-antigen vaccines have an even more complicated process involving multiple steps: tumor biopsy, tumor sequencing, bioinformatics analysis, epitope binding prediction, epitope GMP production and efficient epitope presentation (i.e. sites of injections, adjuvants). All these strategies are labor intensive and expensive. In addition, there remains a high level of uncertainty concerning the identification of what would be the most immunogenic targets for a given cancer/patient. They are also limited in the number of epitopes which can be concomitantly targeted and, therefore, in their ability to generate polyclonal immunity which would address tumor heterogeneity. This approach is further limited by the fact that they rely solely on a single type of antitumor immunity (e.g. HLA-I epitopes, T-cell responses), although other types of immunity might be critical for the overall control of a cancer (HLA-II-mediated responses, B-cell and antibody responses).

Finally, *ex situ* vaccines require an HLA typing of the host which may mean that application of the therapy is restricted to a limited number of patients (e.g. HLA-A2).

Advantages over tumor targeting monoclonal antibodies, CAR-T cells and T-cell engaging bispecific antibodies

Tumor-targeting monoclonal antibodies (TT mAbs), adoptive T-cell therapies (such as chimeric antigen receptor T cells: CAR-T cells) and T-cell engaging bispecific antibodies (TCE mAbs) have the advantage, in theory, of being active despite the absence

Table 3. Intratumoral immunotherapy: universal rather than personalized cancer immunization

	Cancer vaccines	Intratumoral immunotherapy
Therapeutic principle	Tumor-specific molecular targets need to be identified	No antigen (Ag) identification nor isolation required; stimulation against most antigenic tumor epitopes
	Off-target (off tumor) immune stimulation	On-target (intra-lesional) immune stimulation
	Product draining into cutaneous lymph node (or accessible lymph node if intralesional injection)	Product draining into tumor draining lymph node
	Mono- or pauci-clonal T-cell stimulation	Polyclonal T- and B-cell stimulation
Patient eligibility	Peptide vaccines: • Cancer antigen needs to be expressed by the patient's tumor • MHC-I haplotype needs to be compatible with peptide	Injectable tumor lesion available (definition which depends on the expertise of the interventional radiologist)
	Neo-epitope vaccine: • Somatic tumor material (e.g. tumor biopsy) available for mutation analysis • Blood needs to be collected to match for germinal control	No MHC restriction, no pretreatment biopsy required
Drug production	Peptide vaccines: • GMP peptides • Out-licensed adjuvant • Dedicated genetically modified organism facility if encoded into viral vector	Off-the shelf (commercially available products or drugs currently in clinical development)

	Neo-epitope vaccine:
	• Identification of neo-antigen:
	  • tumor DNA/RNA sequencing
	  • HLA-I binding prediction
	  • HLA-I peptide elution
	• GMP production of peptide/DNA/RNA for each patient
	• GMP adjuvants
	• Dedicated GMO facility if encoded into viral vector	

Patient selection	Peptide vaccines: • HLA haplotype compatibility • Target expressed by the tumor (IHC staining on archival or fresh tumor biopsy)	None[a]
	Neo-epitope vaccine: • Identification of neo-antigens with good MHC-I affinity	
Screening period	4–6 weeks if neo-epitope vaccine (production of personalized vaccine)	No delay
Treatment	Sub-cutaneous or into accessible lymph node	Intratumoral

[a]Patients under curative anticoagulants should be switched to low molecular weight heparin. Heparin should be stopped 24 h prior and resumed 24 h after intratumoral or intranodal injection.
IHC, Immuno-Histo-Chemistry.

of HLA expression by the cancer cells. Indeed, they directly target an epitope expressed at the surface of the cancer cells and can induce its destruction by direct or indirect cytotoxicity. Although TT mAbs are generally well tolerated, CAR-T cells and TCE mAbs generate strong cytokine release syndromes in patients and are therefore difficult to implement in a conventional oncology practice. Moreover, TT mAbs, CAR-T cells and TCE mAbs only target a single epitope and therefore they do not address the heterogeneity of the disease and disease relapses/resistance might occur upon selection of an epitope-negative cancer cell subclone [60].

Unlike these strategies, intratumoral immunotherapy offers the opportunity to generate epitope spreading and induce an immune response against both extra- and intracellular antigens. Moreover, it could trigger *de novo* an adaptive antitumor immunity which can better address the heterogeneity (subclones) of the disease that is disseminated throughout the body. Preclinical data have shown that tumor bearing mice treated systemically with a tumor-specific antitumor mAb may eventually relapse with cancer cells which no longer express the targeted epitope. These relapses can be avoided when the systemic antitumor mAb therapy is combined with an intra-tumoral TLR agonist. Such combinations generate an adaptive CD8+ T-cell antitumor immune response which eradicates cancer cells, including those in non-injected tumor lesions [60].

The ideal intratumoral immunotherapy combination

Many reagents can be used for intratumoral immunotherapy, including cytotoxic therapies, pathogen-associated molecular

Table 4. Therapeutic options for intratumoral immunization

Aim	Mean
Tumor antigen release	• Radiotherapy • Oncolytic virus • Tumor targeting mAbs

Enhance tumor antigen presentation	• PRR agonists and analogs
	  • TLR agonists (TLR-3, 4, 7/8, 9)
	  • STING agonists
	  • Oncolytic Virus
	  • Bacteria
	• Anti-CD40 agonistic mAb
	• FLT3-ligand
	• Gene therapy (GM-CSF, FLT3, HSP, CD40L)
	• Dendritic cells

Activate immune effector cells	• Coinhibitory mAbs [anti-PD(L)1, anti-LAG3, anti-KIR, etc.] • Costimulatory mAbs (anti-OX40, anti-CD137, etc.) • Cytokines (PEG-IL-2, IL-12 Mrna, PEG-IL-10) and immunocytokines (CEA-IL2, etc.)
Block/deplete immunosuppressive cells	• Tregs (anti-CTLA4, etc.) • Macrophages (anti-CSF1R, anti-CCR5, etc.)

GMP, generic good manufacturing practice; MHC, major histocompatibility complex; mAb, monoclonal antibody; PRR, pattern recognition receptor.

patterns (PAMP), recombinant viruses, ICT mAbs, gene therapy, DCs and cytokines (Table 4). However, one treatment can exert multiple immune effects within a given tumor. When thinking about intratumoral combinations of immunotherapies, one should aim to address the critical steps that are necessary for efficient T-cell priming (Figure 2). First, some immunogenic cell death should be generated in order to obtain the release of tumor Ags. Such antigen release could be obtained by radiotherapy, cryoablation, radiofrequency, oncolytic agents or tumor targeting mAbs. In case of tumors without immune infiltrates, the recruitment of immune cells is necessary. Both antigen-presenting cells and lymphocytes might be necessary for the induction/enhancement of Ag presentation. Moreover, the clinical success of anti-PD(L)1 mAbs has demonstrated that the blockade/depletion of immunosuppressive cells/pathways is critical for the generation of clinical benefits. This could be achieved by the blockade of co-inhibitory immune check points, the depletion of regulatory T cells (Tregs), or the inhibition of IDO. Also, the stimulation of effector cells via co-stimulatory immune check points can be useful in order to achieve full activity of cytotoxic CD8 T cells, the activation of phagocytosis by macrophages or the enhancement of ADCC/ADCP. Eventually, the expression of MHC molecules by the cancer cells should be required for a proper T-cell-mediated antitumor immunity. Indeed, cancer cell recognition by T cells relies in theory on their membrane expression of MHC molecules. Accordingly, the loss of MHC expression has been related to secondary resistance to anti-PD-1 therapy [61, 62]. Addressing

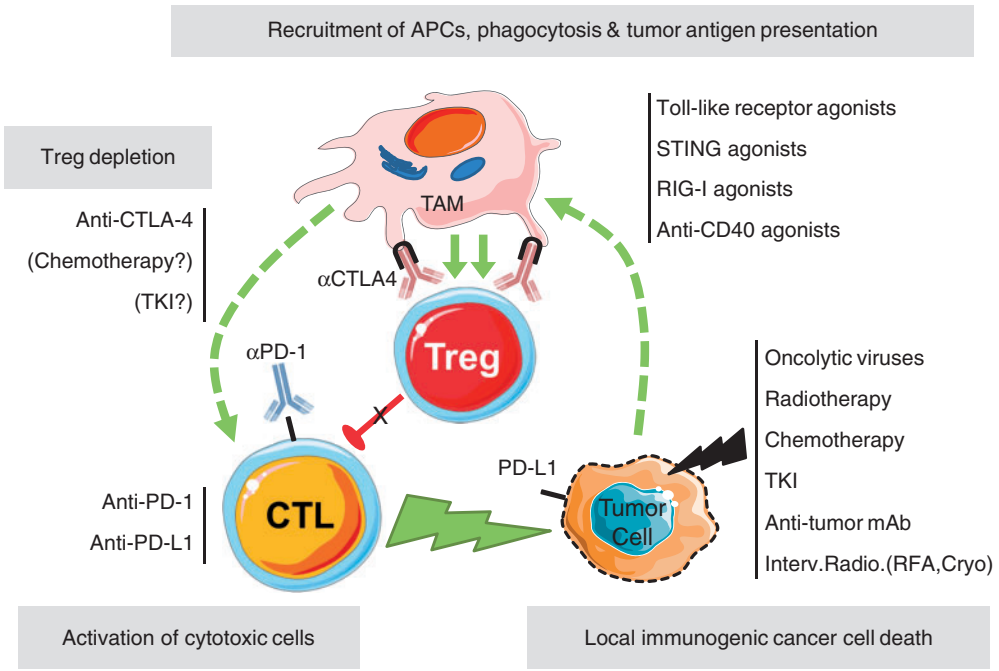

Figure 2. The virtuous circle of intratumoral cancer immunity priming. Local cytotoxic procedures can generate an immunogenic cell death which end-up releasing extra-cellular and intracellular tumor antigens. These procedure can also induce a recruitment of immune cells. Intratumoral injections of immunostimulatory products can also enhance the recruitment of antigen-presenting cells (APCs) and their activation via an upregulation of major histocompatibility complex (MHC) and B7 molecules for the presentation of tumor antigens to the immune system. Some local therapies are also capable of inhibiting or depleting Tregs. Immune checkpoint targeted antibodies can enable the proper activation of cytotoxic antitumor T cells. Besides their local activity, intra-tumoral immunostimulatory therapies also offer the opportunity to act specifically within the draining lymph nodes of a tumor. RFA, radiofrequency ablation; Cryo, cryotherapy.

two or three of these critical steps might be sufficient to generate a virtuous circle. Indeed, tumor cell killing by immune cells may in turn lead to epitope spreading and further enhance the priming phase of the antitumor immune response.

Challenges and future directions

The main criticism often made against intratumoral immunotherapy is about feasibility. However, this appears to be a false challenge. Nowadays, thanks to interventional radiology, endoscopy and laparoscopic surgery, almost every site/organ in the human body can be biopsied and therefore injected. Even CNS tumors have been treated by *in situ* immunization procedures (e.g. TLR 9 agonists) [63]. Therefore, the real challenge is not the intratumoral injection but rather:

- What dose/volume/number of injections/frequency are needed?
- Where to inject: primary tumor versus metastasis? One tumor or several tumors?
- What schedule? Priming versus boosting (repeated treatment) versus prime-boosting (repeated treatment but in a different tumor site)?
- What type of intratumoral immunotherapy for which patient/tumor?

Intratumoral immunotherapy could also be used as a versatile platform to

- Test multiple ($\geq$3) combinations.
- Target multiple immunosuppressive cells (MDSC, Tregs) or factors (IDO, VEGF) concomitantly and with high bioavailability.
- Test different combinations within the same patient: various combinations may be tested in different tumors in the same patient, the patient being his own control (intrapatient comparison). This approach could be used as an *in vivo* test to identify the mechanisms of immune-escape and choose the optimal immunotherapy in a particular patient.
- Explore the heterogeneity of cancer cells and immune responses across sites at the beginning and during the treatment (tumor biopsies taken at every injection).
- Evaluate the impact of tumor DNA mutation burden or the type of genomic abnormalitites (point mutations versus translocations versus indels) on the ability to generate effective antitumor immunity.

Eventually, the scientific results generated through these clinical approaches could build the rationale for personalized immunotherapy. This personalization could be either *via* predictive markers of efficacy or pre-emptive strategies to adapt the treatment to the disease/immunity evolution.

Discussion

Conclusion

Intratumoral immunotherapy is an innovative paradigm which could be helpful to (i) speed up the discovery of biomarkers for resistance/efficacy, (ii) safely evaluate the impact of drugs in the tumor microenvironment and (iii) efficiently assess the synergistic potential of multiple combinations. It offers new opportunities to increase efficacy (local bioavailability, multiple combinations), reduce toxicity (irAE and financial toxicity) and improve our understanding of the antitumor immune responses. Preclinical data, and some clinical results, have shown that intratumoral immunotherapy can overcome resistance to ICT mAbs in multiple tumor types. Eventually, if some combinations of intratumoral immunotherapy turn out to have little abscopal effects but potent local activity, they could be used in a wide spectrum of indications for patients with locally advanced cancers to turn inoperable tumors into operable tumors and prevent subsequent relapses through the generation of a protective antitumor immunity.

Acknowledgement

Figures have been drawn; thanks to the Servier copyright free image bank (http://smart.servier.com/).

Funding

This supplement was sponsored by F. Hoffmann-La Roche.

Disclosure

The four authors are either principal investigators or co-investigators of industry and academic sponsored intratumoral immunotherapy clinical trials.

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