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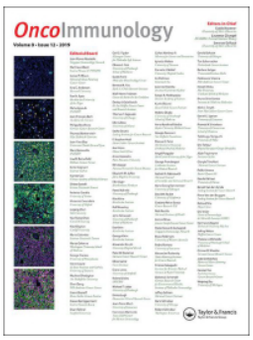

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REVIEW

Trial watch: intratumoral immunotherapy

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ABSTRACT

While chemotherapy and radiotherapy remain the first-line approaches for the management of most unresectable tumors, immunotherapy has emerged in the past two decades as a game-changing treatment, notably with the clinical success of immune checkpoint inhibitors. Immunotherapies aim at (re) activating anticancer immune responses which occur in two main steps: (1) the activation and expansion of tumor-specific T cells following cross-presentation of tumor antigens by specialized myeloid cells (priming phase); and (2) the immunological clearance of malignant cells by these antitumor T lymphocytes (effector phase). Therapeutic vaccines, adjuvants, monoclonal antibodies, cytokines, immunogenic cell death-inducing agents including oncolytic viruses, anthracycline-based chemotherapy and radiotherapy, as well as adoptive cell transfer, all act at different levels of this cascade to (re)instate cancer immunosurveillance. Intratumoral delivery of these immunotherapeutics is being tested in clinical trials to promote superior antitumor immune activity in the context of limited systemic toxicity.

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KEYWORDS
Antitumor immunity; cancer immunosurveillance; immunotherapy; intralesional injection; *in situ* vaccination

Introduction

Brief history of anticancer immunity

In 1890, William B. Coley injected dying bacterial cultures (called Coley toxins) as a treatment for various cancers, including soft tissue sarcoma.[1] In spite of mixed success, this bacterial immunotherapy inspired the application of Bacillus Calmette–Guérin (BCG), an attenuated form of the tuberculosis-causing bacteria, for malignant indications. In 1990, BCG became among the first antitumoral immunotherapy approved by the Food and Drug Administration (FDA) as an adjuvant treatment of superficial bladder carcinoma for which it remains a standard of care to date.[2] In 1994, it was shown that the immune system not only recognizes tumor antigens but also danger signals emitted by cells undergoing stress or abnormal differentiation.[3–5] Despite these elements, the role of the immune system against cancer has long been debated, in part due to the evidence that tumor cells could benefit from a proinflammatory environment, including proliferative and proangiogenic signaling.[6,7] In the early 2000s, the group of Robert Schreiber demonstrated that interferon gamma (IFNG, best known as IFNγ) plays a crucial role in cancer immunosurveillance and that tumors coming from immunodeficient mice are more immunogenic than tumors arising on immunocompetent ones, giving birth to the concept of immunoediting.[8–10] These two fundamental findings led to a regain of interest for cancer immunotherapy, which aims at (re)activating an anticancer immune response.[11,12] At the same time, the evidence came up that some conventional treatments, like anthracycline-based chemotherapy or radiotherapy,[13,14] which were initially used to impair cancer cell proliferation, were also able to induce the release of damage-associated molecular patterns (DAMPs) and antigens, leading to the activation of an adaptive anticancer immune response.[15–18] Nowadays, several immunotherapies are being investigated in oncology: vaccines,[19,20] adjuvants,[21,22] monoclonal antibodies (mAbs),[23,24] virotherapy,[25] cytokines,[26] and adoptive cell transfer.[27,28] Immune checkpoint inhibitors (ICIs) are a subclass of mAbs neutralizing inhibitory immune signals such as programmed cell death 1 (PDCD1, best known as PD1), CD174 (best known as PDL1), or cytotoxic T lymphocyte-associated protein 4 (CTLA4).[29,30] Eight ICIs are currently on the market after demonstrating remarkable antineoplastic efficacy first in melanoma, then in a still growing number of cancer histotypes (Table 1).[31–33]

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Table 1. Cancer immunotherapeutics approved by the FDA.

Type of immunotherapy	Subtype of immunotherapy	Name of the agent	Indication	Injection route
Adpotive cell tranfer	CAR-T cells bearing a CD19-specific TCR	Axicabtagene ciloleucel	B lymphoma	*i.v.*
		Brexucabtagene autoleucel	B lymphoma	
		Lisocabtagene maraleucel	B lymphoma	
		Tisagenlecleucel	B leukemia, B lymphoma	
	CAR-T cells bearing a BCMA-specific TCR	Idecabtagene vicleucel	Multiple myeloma	
Cytokine	IL2 analog	Aldesleukin	Kidney cancer, Melanoma	*i.v.*
	IFNα analog	IFNα2a (Roferon-A)	Leukemia, Sarcoma	*s.c.* or *i.m.*
		IFNα2b (Intron A)	Leukemia, Lymphoma, Melanoma, Sarcoma	*s.c.* or *i.v.*
		Peg-IFNα2b (PegIntron)	Melanoma	*s.c.*
mAb – Immune checkpoint antagonist	Anti-CTLA4	Ipilimumab	CRC, Kidney cancer, Liver cancer, Lung cancer, Lymphoma, Melanoma, Squamous cell carcinoma, Urothelial cancer	*i.v.*
	Anti-PD1	Cemiplimab	Basal cell carcinoma, Cutaneous squamous cell carcinoma, Lung cancer	
		Dostarlimab	Endometrial cancer	
		Nivolumab	CRC, Gastroesophageal cancer, Kidney cancer, Liver cancer, Lung cancer, Lymphoma, Melanoma, Mesothelioma, HNSCC, Urothelial cancer	
		Pembrolizumab	Breast cancer, Cervical cancer, CRC, Endometrial carcinoma, Gastroesophageal cancer, HNSCC, Liver cancer, Lung cancer, Lymphoma, Kidney cancer, Melanoma, Merkel cell carcinoma, Urothelial cancer	
	Anti-PDL1	Atezolizumab	Breast cancer, Lung cancer, Urothelial cancer	
		Avelumab	Kidney cancer, Merkel cell carcinoma, Urothelial cancer	
		Durvalumab	Lung cancer, Urothelial cancer	
mAb – Tumor antigen-targeted	Anti-CD19	Tafasitamab	B lymphoma	*i.v.*
	Anti-CD20	Obinutuzumab	Leukemia, Lymphoma	
		Ofatumumab	Leukemia	
		Rituximab	Leukemia, Lymphoma	
	Anti-CD38	Daratumumab	Multiple myeloma	
		Isatuximab	Multiple myeloma	
	Anti-EGFR	Cetuximab	CRC, HNSCC	
		Necitumumab	Lung cancer	
		Panitumumab	CRC	
	Anti-GD2	Dinutuximab	Pediatric neuroblastoma	
		Naxitamab	Neuroblastoma	
	Anti-HER2	Margetuximab	Breast cancer	
		Pertuzumab	Breast cancer	
		Trastuzumab	Breast cancer, Gastroesophageal cancer	*i.v.* or *s.c.*
	Anti-PDGFRα	Olaratumumab	Sarcoma	*i.v.*
	Anti-SLAMF7	Elotuzumab	Multiple myeloma	
	Anti-VEGFR2	Ramucirumab	CRC, Gastroesophageal cancer, Liver cancer, Lung cancer	
mAb – Cytokine neutralization	Anti-VEGF	Bevacizumab	Cervical cancer, CRC, Glioblastoma, Kidney cancer, Ovarian and fallopian cancer, Peritoneal cancer	*i.v.*
mAb – Bispecific	Anti-EGF and anti-MET	Amivantamab	Lung cancer	*i.v.*
	Anti-CD19 and anti-CD3	Blinatumomab	B leukemia	

(*Continued*)

Table 1. (Continued).

Type of immunotherapy	Subtype of immunotherapy	Name of the agent	Indication	Injection route
mAb – Conjugated	Anti-BCMA conjugated with auristatin F	Belantamab mafodotin	Multiple myeloma	*i.v.*
	Anti-CD30 conjugated with auristatin E	Brentuximab vedotin	Lymphoma	
	Anti-Nectin-4 conjugated with auristatin E	Enfortumab vedotin	Urothelial carcinoma	
	Anti-CD33 conjugated with N-acetyl-gamma calicheamicin	Gemtuzumab ozogamicin	Leukemia	
	Anti-CD20 conjugated with isotype yttrium 90	Ibritumomab tiuxetan	B lymphoma	
	Anti-CD22 conjugated with N-acetyl gamma calicheamicin	Inotuzumab ozogamicin	B leukemia	
	Anti-CD19 conjugated with pyrrolobenzodiazepine dimer	Loncastuximab tesirine	B lymphoma	
	Anti-CD22 conjugated with *Pseudomonas* exotoxin	Moxetumomab pasdotox	Leukemia	
	Anti-CD79B conjugated with auristatin E	Polatuzuman vedotin	B lymphoma	
	Anti-TACSTD2 conjugated with SN-38 (active metabolite of irinotecan)	Sacituzumab govitecan	Breast cancer, Urothelial cancer	
	Anti-HER2 conjugated with deruxtecan	Trastuzumab deruxtecan	Breast cancer, Gastroesophageal cancer	
	Anti-HER2 conjugated with DM1	Trastuzumab emtansine	Breast cancer	
mAb – Other	Anti-RANKL	Denosumab	Bone cancer	*s.c.*
Nonpathogenic bacterium	Live attenuated strain of *Mycobacterium Bovis* (TLR2/4 agonist)	Bacillus Calmette-Guérin	Urothelial cancer	*i.t.*
Oncolytic virus	Recombinant Herpes simplex virus 1	Talimogene laherparepvec	Melanoma	*i.t.*
PRR agonist	TLR7 agonist	Imiquimod	Basal cell carcinoma	*topical*
Therapeutic vaccine	Autologous DCs presenting the antigen PAP fusioned to GM-CSF	Sipuleucel-T	Prostate cancer	*i.v.*

Source: https://www.fda.gov/. List updated on 08/06/2021. BCMA, B cell maturation antigen; CAR-T, chimeric antigen receptor T cell; CRC, colorectal cancer; DAMP, damage-associated molecular pattern; DC, dendritic cell; HNSCC, head and neck squamous cell carcinoma; ICI, immune checkpoint inhibitor; *i.m.*, intramuscular; *i.t.*, intratumoral; *i.v.*, intravenous; mAb, monoclonal antibody; PRR, pattern recognition receptor; *s.c.*, subcutaneous.

Mechanisms of anticancer immunity

During tumorigenesis, tumor cells require sustained blood influx and stromal reorganization to support their expansion.[34] This environment promotes proinflammatory cytokine release by malignant and stromal cells.[35,36] Such cytokines include tumor necrosis factor (TNF), transforming growth factor beta 1 (TGFB1, best known as TGFβ), interleukin 1 beta (IL1B, best known as IL1β), IL6 and IL10, altogether leading to the recruitment of natural killer (NK) cells, NKT cells, macrophages and dendritic cells (DCs) at the tumor site.[3,37–39] In turn, recruited immune cells secrete proinflammatory cytokines, like IL12 and IFNγ.[40] Tumor-infiltrating NK cells mediate cytotoxic effect on neoplastic cells promoting the release of tumor antigens.[41–44] Dying cancer cells and released antigens are ingested by DCs, which undergo activation upon sensing of DAMPs via their pattern recognition receptors (PRRs).[45,46] Ultimately, these signals promote the maturation of DCs, in particular the $XCR1^+CLEC9a^+$ conventional DC subtype 1 (cDC1),[47] which is characterized by the cross-presentation of tumor antigens as well as the expression of co-stimulatory molecules and the secretion of proinflammatory cytokines including IL12, IL6, TNF, and type I IFNs (IFN-I).[48–50] Activated DCs migrate to the draining lymph node where they drive naïve $CD4^+$ T cells into the type 1 helper T (T_H1) cell lineage.[51–53] DCs also prime naïve $CD8^+$ T cells and promote their differentiation into cytotoxic T lymphocytes (CTLs).[51,52,54] Similar to NK cells, expanded CTLs can reach the tumor bed via the bloodstream.[43] There, they elicit cytotoxicity against the cancer cells which harbor their cognate antigen, mostly through secretion of perforins, granzymes, and granulysins, as well as via the expression of pro-apoptotic receptors like TNF-related apoptosis inducing ligand (TRAIL) or Fas ligand (FASLG).[55–58]

When tumors have undergone immunoediting and escaped from these anticancer immune mechanisms, a clinically detectable neoplastic mass arises.[59] At this stage, the composition of the tumor immune infiltrate influences the outcome of the disease, with the presence of CTLs, among others, as good prognostic factors.[60–62] Importantly, the nature and/or density of the tumor-infiltrating populations of leukocytes can be modified by immunotherapy. Immunotherapeutic approaches aim at (re)activating an anticancer immune response, either by enhancing cancer adjuvanticity and/or antigenicity, or by providing some immune cells which harbor cytotoxic functions. Immunotherapeutic interventions that have been approved or which are under clinical evaluation for oncological indications include: [i] some agonists of PRRs like Toll-like receptors (TLRs),[63,64] or of their adapters like stimulator of interferon response cGAMP interactor 1 (STING1),[65–67] [ii] some agents inducing cancer immunogenic cell death (ICD) like oncolytic viruses (OVs) and some chemotherapeutic agents,[68–70] [iii] a plethora of mAbs which targets surface tumor antigens and mediates cytotoxicity,[71] [iv] other mAbs belong to the subclass of immune checkpoint modulators and support CTL activation either by blocking co-inhibitory immune signals, or by stimulating co-stimulatory ones at the surface of malignant and/or stromal entities,[72] [v] vaccines which aim at feeding DCs with enlarged amounts of tumor antigens to further stimulate T cell priming,[73] [vi] adoptive transfer of lymphocytes with tumor killing activity (e.g. NK cells, CTLs),[74] and finally [vii] cytokines which can promote an inflammatory environment and participate in immune cell recruitment and activation (Figure 1; Table 1).[75,76]

Intratumoral versus systemic injection

Systemic immunotherapies have demonstrated their efficacy to induce durable antitumor immune responses and to increase overall survival (OS) in several solid cancers.[77] Yet, a majority of patients experience adverse events which can lead to treatment disruption. Moreover, the immunosuppressive tumor microenvironment efficiently shields off infiltration by cellular mediators of cancer immunosurveillance, thus frequently resulting in the inefficiency of systemic immunotherapies. Preclinical studies have demonstrated that local delivery of immunostimulatory products, such as OVs, cytokines, and PRR agonists could overcome the resistance to systemic immune checkpoint blockade therapies.[78–81] These observations have precipitated the evaluation of intratumoral (*i.t.*) immunotherapy in the clinic.[82,83] This dynamic has been supported in 2015 by the FDA approval of talimogene laherparepvec (T-VEC) for *i.t.* oncolytic virotherapy of melanoma.[84,85]

The intralesional route offers several advantages over systemic infusion. First, local administration translates into an immediate effect of the drugs on targeted cancer cells. Consequently, lower dosages could be applied without impairing therapeutic efficacy.[86] Meanwhile, it reduces treatment cost and opens some opportunities for combinations of drugs. Furthermore, by limiting systemic exposure, off-target toxicity is attenuated, especially inflammation and autoimmunity which are often observed after *i.v.* injection of immunotherapies. Additionally, in the case of ICD-inducing agents, their topical delivery favors enhanced cytotoxicity and potent release of the tumor antigen repertoire. In this scenario, tumor cells stressed/dying upon treatment work as vaccines and induce a polyclonal immune response. This both diminishes the probability to observe resistance and leverages long-term and systemic protection (responsible for the so-called "abscopal effect").[87–90] Moreover, tumors which show a low immune infiltration (e.g. bone and ovary cancers), and do not receive a sufficient dose of drugs after systemic injection, could benefit from a topical administration.[91]

For the purpose of this review, we focused on clinical trials involving intratumoral immunotherapy that were initiated during the past 3 y (January 2018–June 2021). Of note, complementary review articles have been published on the topic recently.[92–94] Out of 153 clinical trials matching our query on clinicaltrials.gov, 64 are investigating virotherapies, 36 PRR agonists, 17 monoclonal antibodies, 7 cytokines, 5 ICD-inducing synthetic agents, 4 adoptive transfer of lymphocytes, 4 therapeutic vaccines, 2 semi-synthetic adjuvants, 1 attenuated bacterial strain, and 14 a combination of different immunotherapeutic strategies (Figure 2, Table 2). In the following sections, clinical investigations have been reported according to the immunotherapic strategy involved.

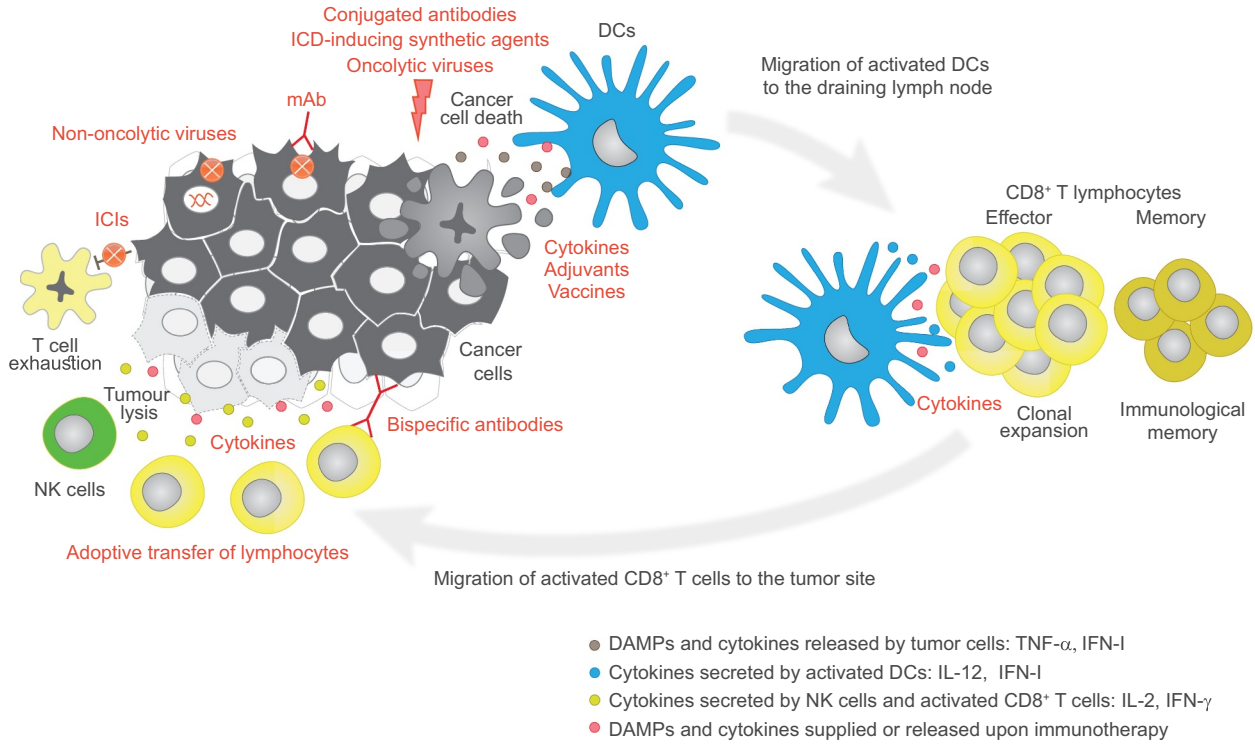

Figure 1. Types of immunotherapies and their targets in the cancer-immunity cycle. Upon immunogenic cell death of cancer cells, dendritic cells (DCs) are recruited to the lesion where they uptake and present tumor antigens to naïve $CD8^+$ T cells, triggering their differentiation into cytotoxic $CD8^+$ T cells which, together with natural killer (NK) cells, eliminate cancer cells. Memory T cells are also generated during this process. Cancer cells may express immune checkpoint ligands contributing to T cell exhaustion. Immunotherapeutics (in red) can act at different levels of the (re)establishment of this anticancer immune response. DAMP, damage-associated molecular pattern; DC, dendritic cell; ICD, immunogenic cell death; ICI, immune checkpoint inhibitor; IFN, interferon; IFN-I, type 1 interferons; IL, interleukin; mAb, monoclonal antibody; TNF, tumor necrosis factor.

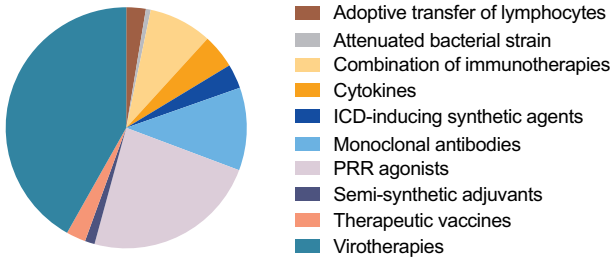

Figure 2. Types of intratumoral immunotherapy across recently initiated clinical trials. Pie chart depicting the proportion of each type of immunotherapeutic interventions in recently initiated clinical investigations. ICD, immunogenic cell death.

Antibodies

Therapeutic mAbs can either (a) directly bind to surface markers on malignant cells or on their stromal accomplices (e.g. HER2 in some breast cancers), (b) target receptors on leukocytes (e.g. PD1 on T lymphocytes),[95] or (c) neutralize some trophic factors which support tumor growth (e.g. vascular endothelial growth factor [VEGF]), in order to precipitate the elimination of the targeted tumor or tumor-supporting cells, to sustain anticancer immunity, and/or to inhibit protumor pathways. Therapeutic mAbs can be sorted into three main categories, namely naked, conjugated and bispecific antibodies.

[i] *Naked antibodies*. Some naked mAbs act by promoting antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cell-mediated cytotoxicity (ADCC), or complement-dependent cytotoxicity (CDC).[96–98] For instance, rituximab which binds to CD20 mediates the killing of B lymphoma cells through such mechanisms.[99] Other naked mAbs contribute to cancer cell elimination by exerting either an agonistic or an antagonistic activity on the targeted receptor. For instance, binding of agonistic anti-CD40 to CD40 at the surface of antigen-presenting cells triggers a co-stimulatory signal which promotes maturation of dendritic cells and supports the priming of tumor-specific T cells.[100–102] By contrast, binding of antagonistic anti-PD1 to PD1 at the surface of T lymphocytes blocks its interaction with the co-inhibitory immune checkpoint PDL1 at the tumor cell membrane, and thus allows sustained effector T cell activity.[17,103] Each ICI aims to interfere with signals associated with cancer immune evasion, and prevent or revert inactivation/exhaustion of T cells responsible for antitumor immunity.[17] Yet, in the case of ipilimumab, binding to its immunoinhibitory target CTLA4

Table 2. Clinical trials involving intratumoral immunotherapies started between January 2018 and June 2021.

Single type of *i.t.* immunotherapy							
Single type of *i.t.* immunotherapy	Subtype of *i.t.* immunotherapy	Agent for *i.t.* immunotherapy	Non-*i.t.* co-therapy[*i.t.* co-therapy]	Indications	Trial status	Phases	NCT Number
Adoptive transfer of lymphocytes	NK cells	NK cells	-	Glioma	Not yet recruiting	I	NCT04254419
		CYNK001	-	Astrocytoma, Glioblastoma	Active, not recruiting	I	NCT04489420
	CAR-T cells	HER2(EQ)BBzeta/CD19t^{+} T cells	-	Glioma	Recruiting	I	NCT03389230
		Nectin4/FAP-targeted CAR-T cells (expressing IL7 and CCL19, or IL12)	-	Nectin4-positive solid tumors	Recruiting	I	NCT03932565
Cytokine	GM-CSF	N/D	Pembrolizumab, Cryoimmunotherapy, Imiquimod	Breast cancer	Terminated	I	NCT03982004
	GM-CSF + IFNα2b + IL12 single chain + IL15sushi (saline-formulated mixture of 4 mRNAs)	SAR441000 (BNT131)	Cemiplimab	Solid tumors	Recruiting	I	NCT03871348
	IL2	Aldesleukin	-	Melanoma	Recruiting	III	NCT03233828
	IL12 (plasmid)	Tavokinogene telseplasmid + electroporation (Tavo-EP)	Nivolumab	Melanoma	Recruiting	II	NCT04526730
			Pembrolizumab, Epacadostat	HNSCC	Recruiting	II	NCT03823131
			Pembrolizumab, Nab-paclitaxel	TNBC	Recruiting	II	NCT03567720
	IL12 (mRNA encapsulated in lipid nanoparticles)	MEDI1191	Durvalumab	Solid tumors	Recruiting	I	NCT03946800
ICD-inducing synthetic agent (confirmed or candidate)	Chemotherapy	Cisplatin	-	NSCLC	Recruiting	I	NCT04809103
		INT230-6	Surgery	Breast cancer	Recruiting	II	NCT04781725
		All or subsets of the following drugs: Cisplatin, Carboplatin, Cyclophosphamide, Doxorubicin, Gemcitabine, Paclitaxel, Pemetrexed, Vinorelbine	[All or subsets of the following drugs: Etoposide, Ifosfamide, Niraparib, Olaparib, Rucaparib, Topotecan], Surgery	Fallopian tube cancer, Ovarian cancer, Peritoneal cancer	Recruiting	I	NCT04701645
	Oncolytic peptide	CyPep-1	-	Advanced solid tumors	Recruiting	I/II	NCT04260529
		LTX-315	Pembrolizumab	Advanced solid tumors	Not yet recruiting	II	NCT04796194
mAb – Immune checkpoint antagonist	Anti-CD39	SRF617	Gemcitabine, Nab-paclitaxel, Pembrolizumab	Solid tumors	Recruiting	I	NCT04336098
	Anti-PD1	Cemiplimab	Surgery	CSCC	Completed	I	NCT03889912
		Nivolumab	-	Kaposi sarcoma	Active, not recruiting	I	NCT03316274
	Anti-PD1 ± Anti-CTLA4	Ipilimumab, Nivolumab	Cyclophosphamide, Cryosurgery	Prostate cancer	Recruiting	II	NCT04090775
		Ipilimumab, Pembrolizumab	-	Breast cancer, Cervical cancer, CRC, HNSCC, Liver cancer, Lung cancer, Melanoma, Ovarian cancer, Pancreatic cancer, Renal cancer	Recruiting	II/III	NCT03755739
mAb – Immune checkpoint agonist	Anti-CD40	2141 V-11	-	Melanoma, Other solid tumors	Recruiting	I	NCT04059588
		ABBV-927	-	HNSCC	Terminated	I	NCT03818542
		rAd.CD40L	Pembrolizumab	Melanoma	Withdrawn	I/II	NCT02719015
		Selicrelumab	Atezolizumab	B-cell non-Hodgkin lymphoma	Terminated	I	NCT03892525

(*Continued*)

Table 2. (Continued).

Single type of *i.t.* immunotherapy							
Single type of *i.t.* immunotherapy	Subtype of *i.t.* immunotherapy	Agent for *i.t.* immunotherapy	Non-*i.t.* co-therapy[*i.t.* co-therapy]	Indications	Trial status	Phases	NCT Number
	nti-CD137	Urelumab	Nivolumab	Solid tumors	Not yet recruiting	I/II	NCT03792724
mAb – Conjugated	Anti-EDB-FN fused to IL2 or TNFα	L19IL2 + L19TNF	-	Non-melanoma skin cancers	Recruiting	II	NCT04362722
	Anti-GD2 fused to IL2	IT-hu14.18-IL2 (EMD 273063)	Radiotherapy, Ipilimumab, Nivolumab	Melanoma	Recruiting	I/II	NCT03958383
	Anti-mesothelin conjugated with *Pseudomonas* exotoxin	LMB-100	Ipilimumab	Mesothelioma	Recruiting	I	NCT04840615
	SIRPα-Fc-CD40L	SL-172154	-	CSCC, HNSCC	Recruiting	I	NCT04502888
mAb – Mixed	Anti-CD20/Anti-CD38/Anti-PD1/Anti-HER2	Daratumumab, Nivolumab, Obinutuzumab, Pembrolizumab, Rituximab, Trastuzumab	[Belinostat, Carfilzomib, Gemcitabine, Romidepsin]	Breast cancer, Lymphoma	Suspended	I	NCT03432741
	Anti-CD40 + anti-EGFR conjugated with *Pseudomonas* exotoxin	2141-V11, D2C7	-	Glioma	Recruiting	I	NCT04547777
	(Anti-ILT3/Ani-ILT4) + Anti-PD1	MK-0482, MK-4830, Pembrolizumab	Surgery	Solid tumors	Not yet recruiting	I	NCT04541108* (Exp. arm 3)
Nonpathogenic bacterium	Attenuated Clostridium	*C. novyi*-NT	Pembrolizumab, Doxycycline	Breast cancer, Endocrine cancer, Gastrointestinal cancer, Genital cancer, Lung cancer, Nervous system cancer, Sarcoma, Urothelial cancer	Recruiting	I	NCT03435952
Oncolytic viruses	Adenovirus	AdAPT-001	-	Solid tumors	Recruiting	I	NCT04673942
		CAdVEC	HER2-specific CAR T cell	HER-2-positive solid tumors	Recruiting	I	NCT03740256
		DNX-2440	Surgery	Breast cancer, CRC, Kidney cancer, Liver cancer, Gastrointestinal cancer, Melanoma, Periampullary cancer, Sarcoma, SCC	Recruiting	I	NCT04714983
		LOAd703	Chemotherapy (standard-of-care, gemcitabine)	Biliary cancer, CRC, Ovarian cancer, Pancreatic cancer	Recruiting	I/II	NCT03225989
		ONCOS-102	Cyclophosphamide, DCVac/Pca	Prostate cancer	Terminated	I/II	NCT03514836
		ORCA-010	-	Prostate cancer	Recruiting	I/II	NCT04097002
		Telomelysin (OBP-301)	Carboplatin, Paclitaxel, Radiotherapy	Gastroesophageal cancer	Recruiting	I	NCT04391049
			Pembrolizumab, Radiotherapy	HNSCC	Recruiting	II	NCT04685499
	Herpesvirus	HSV G207	Radiotherapy	Glioma	Not yet recruiting	II	NCT04482933
		OH2	Cisplatin, Fluorouracil, LP002	Digestive system cancer	Recruiting	I	NCT04755543
		ONCR-177	Pembrolizumab	Solid tumors	Recruiting	I	NCT04348916
		OrienX010	Toripalimab	Melanoma	Active, not recruiting	I	NCT04197882
			JS001	Melanoma	Recruiting	I	NCT04206358
		RP1	Cemiplimab	SCC	Recruiting	II	NCT04050436
			-	SCC	Recruiting	I	NCT04349436
		RP3	Undefined anti-PD1	Visceral tumors	Recruiting	I	NCT04735978
		T-VEC	Atezolizumab	CRC, TNBC	Active, not recruiting	I	NCT03256344
			-	Non-melanoma skin cancer	Recruiting	I	NCT03458117
			Atezolizumab	Breast cancer	Recruiting	I	NCT03802604
			Pembrolizumab, Surgery	Melanoma	Recruiting	II	NCT03842943
			-	Angiosarcoma of skin	Active, not recruiting	II	NCT03921073

(*Continued*)

Table 2. (Continued).

Single type of *i.t.* immunotherapy							
Single type of *i.t.* immunotherapy	Subtype of *i.t.* immunotherapy	Agent for *i.t.* immunotherapy	Non-*i.t.* co-therapy[*i.t.* co-therapy]	Indications	Trial status	Phases	NCT Number
			-	Kaposi sarcoma	Not yet recruiting	II	NCT04065152
			Pembrolizumab	Melanoma	Active, not recruiting	II	NCT04068181
			Nivolumab, Surgery	Melanoma	Recruiting	II	NCT04330430
			Surgery	Melanoma	Recruiting	II	NCT04427306
			Radiotherapy	Sarcoma	Recruiting	I/II	NCT04599062
		VG161	-	Solid tumors	Recruiting	I	NCT04758897
			-	Liver cancer	Recruiting	I	NCT04806464
	Paramyxovirus	MV-s-NAP	-	Breast cancer	Recruiting	I	NCT04521764
		TMV-018	5-fluorocytosine, Undefined anti-PD1	Gastrointestinal cancer	Withdrawn	I	NCT04195373
	Picornavirus	V937 (Coxsackievirus A21)	Pembrolizumab	Melanoma	Recruiting	II	NCT04152863
			Pembrolizumab	Gastric cancer, Liver cancer, SCC, TNBC, Other solid tumors	Recruiting	I/II	NCT04521621
		PVSRIPO	Surgery	Breast cancer	Recruiting	I	NCT03564782
			-	Melanoma	Active, not recruiting	I	NCT03712358
			Pembrolizumab	Brain tumors	Recruiting	II	NCT04479241
			Nivolumab	Melanoma	Withdrawn	I	NCT04125719
	Poxvirus	ASP9801	-	(Sub)cutaneous tumors, Visceral tumors	Recruiting	I	NCT03954067
		BT-001	Pembrolizumab	Melanoma, Merkell cell carcinoma, NSCLC, Sarcoma, TNBC	Recruiting	I/II	NCT04725331
		OVV-01	Atezolizumab, Pembrolizumab	Solid tumors	Active, not recruiting	I	NCT04787003
		Pexa-Vec (JX-594)	Cemiplimab	Kidney cancer	Recruiting	I/II	NCT03294083
			ZKAB001	Melanoma	Not yet recruiting	I/II	NCT04849260
		TBio-6517	Pembrolizumab	CRC, TNBC, Other solid tumors	Recruiting	I/II	NCT04301011
	Rhabdovirus	VSV-IFNbetaTYRP1	VSV-IFNbetaTYRP1	Melanoma	Recruiting	I	NCT03865212
		Voyager V1	Cemiplimab, Voyager V1	Melanoma	Recruiting	II	NCT04291105
		MG1-E6E7	Ad-E6E7, Atezolizumab, MG1-E6E7	HPV-associated cancers	Active, not recruiting	I	NCT03618953
		MG1-MAGEA3	Ad-MAGEA3, Cyclophosphamide, MG1-MAGEA3, Pembrolizumab	Melanoma, SCC	Withdrawn	I	NCT03773744
	Poxvirus	ASP9801	-	(Sub)cutaneous tumors, Visceral tumors	Recruiting	I	NCT03954067
		BT-001	Pembrolizumab	Melanoma, Merkell cell carcinoma, NSCLC, Sarcoma, TNBC	Recruiting	I/II	NCT04725331
		OVV-01	Atezolizumab, Pembrolizumab	Solid tumors	Active, not recruiting	I	NCT04787003
		Pexa-Vec (JX-594)	Cemiplimab	Kidney cancer	Recruiting	I/II	NCT03294083
			ZKAB001	Melanoma	Not yet recruiting	I/II	NCT04849260
		TBio-6517	Pembrolizumab	CRC, TNBC, Other solid tumors	Recruiting	I/II	NCT04301011
Other virus-based immunotherapies	Adenovirus	Ad-P53	Nivolumab	Solid tumors	Terminated	I/II	NCT02842125
			Atezolizumab, Durvalumab, Nivolumab, Pembrolizumab	Lymphoma, Solid tumors	Recruiting	II	NCT03544723
		Ad-RTS-hIL12	Veledimex, Surgery	Glioblastoma	Active, not recruiting	I	NCT03679754

(*Continued*)

Table 2. (Continued).

Single type of *i.t.* immunotherapy							
Single type of *i.t.* immunotherapy	Subtype of *i.t.* immunotherapy	Agent for *i.t.* immunotherapy	Non-*i.t.* co-therapy[*i.t.* co-therapy]	Indications	Trial status	Phases	NCT Number
			Veledimex, Nivolumab, Surgery	Glioblastoma	Active, not recruiting	I	NCT03636477
			Veledimex, Cemiplimab, Surgery	Glioblastoma	Active, not recruiting	II	NCT04006119
		MTG201	Nivolumab	Mesothelioma	Recruiting	II	NCT04013334
	Arenavirus	HB-201	HB-201, HB-202	HPV16-positive HNSCC	Recruiting	I/II	NCT04180215
			Chemoradiation	HPV16-positive oropharynx cancer, HPV16-positive cervical cancer	Recruiting	I	NCT04630353
	Flavivirus	PV-001-DV	PV-001-DC	Melanoma	Not yet recruiting	I	NCT03990493
	Orthomyxovirus	Unattenuated influenza vaccine	Surgery	CRC	Recruiting	II	NCT04591379
		Quadrivalent inactivated influenza vaccine	Ipilimumab, Nivolmab, Pembrolizumab, Quadrivalent inactivated influenza vaccine, Surgery	Melanoma	Not yet recruiting	I	NCT04697576
	Poxvirus	MVA-BN-Brachyury	Atezolizumab, PROSTVAC, Surgery	Prostate cancer	Withdrawn	II	NCT04020094
PRR agonist	RIG-1 agonist	MK-4621	Pembrolizumab	Solid tumors	Completed	I	NCT03739138
	STING agonist	ADU-S100 (MIW815)	Pembrolizumab	HNSCC	Active, not recruiting	II	NCT03937141
		BI 1387446	Ezabenlimab	Neoplasms	Recruiting	I	NCT04147234
		E7766	-	Lymphoma, Solid tumors	Recruiting	I	NCT04144140
		IMSA101	Undefined ICIs	Solid tumors	Recruiting	I/II	NCT04020185
		MK-1454	Pembrolizumab	HNSCC	Active, not recruiting	II	NCT04220866
		SYNB1891	Atezolizumab	Lymphoma, Solid tumors	Recruiting	I	NCT04167137
	TLR3 agonist	BO-112	Nivolumab, Radiotherapy, Surgery	Sarcoma	Recruiting	I	NCT04420975
			Pembrolizumab	Liver cancer	Not yet recruiting	Early I	NCT04777708
			Pembrolizumab	CRC, Gastroesophageal cancer	Recruiting	II	NCT04508140
			Pembrolizumab	Melanoma	Recruiting	II	NCT04570332
		Poly-ICLC (Hiltonol)	Surgery	Mesothelioma	Recruiting	I	NCT04525859
	TLR4 agonist	G100	-	Lymphoma	Withdrawn	II	NCT03742804
	TLR7 agonist	LHC 165	PDR001	Solid tumors	Active, not recruiting	I	NCT03301896
	TLR7/8 agonist	NKTR-262	Bempegaldesleukin, Nivolumab	Breast cancer, CRC, HNSCC, Skin cancers, Sarcoma	Active, not recruiting	I/II	NCT03435640
		TransCon	Pembrolizumab	Solid tumors	Recruiting	I/II	NCT04799054
	TLR8 agonist	Motolimod (VTX-2337)	Nivolumab, Surgery	HNSCC	Recruiting	I	NCT03906526
	TLR9 agonist	Cavrotolimod (AST-008)	Cemiplimab, Pembrolizumab	Skin cancers, Other solid tumors	Recruiting	I/II	NCT03684785
		CMP-001	Atezolizumab, Radiotherapy	NSCLC	Completed	I	NCT03438318
			Cemiplimab	Skin cancers, TNBC	Not yet recruiting	II	NCT04916002
			Ipilimumab, Nivolumab, Radiotherapy	CRC, Other solid tumors	Unknown status	I	NCT03507699
			Nivolumab, Surgery	Melanoma, Lymph node cancer	Active, not recruiting	II	NCT03618641
			Nivolumab, Surgery	Melanoma	Recruiting	II	NCT04401995
			Nivolumab	Melanoma	Recruiting	II/III	NCT04695977
			Nivolumab	Melanoma	Recruiting	II	NCT04698187
			Pembrolizumab	Lymphoma	Recruiting	I/II	NCT03983668
			Pembrolizumab	HNSCC	Recruiting	II	NCT04633278

(*Continued*)

Table 2. (Continued).

Single type of *i.t.* immunotherapy

Single type of *i.t.* immunotherapy	Subtype of *i.t.* immunotherapy	Agent for *i.t.* immunotherapy	Non-*i.t.* co-therapy[*i.t.* co-therapy]	Indications	Trial status	Phases	NCT Number
			Pembrolizumab, Surgery	Melanoma	Recruiting	II	NCT04708418
			Radiotherapy	TNBC	Not yet recruiting	II	NCT04807192
		SD-101	Epacadostat, Radiotherapy	Solid tumors, Lymphoma	Active, not recruiting	I/II	NCT03322384
			Nivolumab, Radiotherapy	Pancreatic cancer	Recruiting	I	NCT04050085
			Abiraterone acetate, Leuprolide acetate, Pembrolizumab, Prednisone, Radiotherapy	Prostate cancer	Recruiting	II	NCT03007732
		Tilsotolimod (IMO-2125)	ABBV-181, ABBV-368, Nab-paclitaxel	HNSCC	Recruiting	I	NCT04196283
			Ipilimumab	Metastatic Melanoma	Active, not recruiting	III	NCT03445533
			Ipilimumab, Nivolumab	Solid tumors	Recruiting	II	NCT03865082
		Undefined CpG ODN	Electroporation, FOLFIRINOX, Nivolumab	Pancreatic cancer	Recruiting	I	NCT04612530
Semi-synthetic adjuvants	Biopolymer	Copaxone (glatiramer acetate)	Surgery	Skin cancers	Recruiting	I	NCT03982212
		IP-001 (N-dihydrogalactochitosan)	Thermal ablation	Solid tumors	Recruiting	I/II	NCT03993678
Therapeutic vaccine	DC-based	Ad-CCL21-DC	Pembrolizumab	NSCLC	Recruiting	I	NCT03546361
		DCVax-Direct	-	Breast cancer, Lung cancer	Unknown status	I	NCT03638765
	DNA-based	IFx-Hu2.0	-	Melanoma	Active, not recruiting	I	NCT03655756
			-	Merkel cell carcinoma, SCC	Recruiting	I	NCT04160065

Mixed types of *i.t.* immunotherapy

Types of *i.t.* immunotherapy	Subtypes of *i.t.* immunotherapy	Agents for *i.t.* immunotherapy	Non-*i.t.* co-therapy[*i.t.* co-therapy]	Indications	Trial status	Phases	NCT Number
Adoptive transfer of CAR-T cells + Chemotherapy + PRR agonist	CAR-T cells + ICD chemoinducers (?) + TLR9 agonist	CAR-T cells secreting scFv against OX40, Drug-eluting beads, CpG ODN	Microwave ablation	Lung cancer, Liver cancer	Recruiting	I	NCT04952272
Adoptive transfer of DCs + mAb	DCs + Anti-CTLA4 + Anti-PDL1	CD1c(BDCA-1)+ myeloid DCs, Ipilimumab, Avelumab	Nivolumab	Solid tumors	Completed	I	NCT03707808
	DCs + Anti-CTLA4 + Anti-PDL1	CD1c(BDCA-1)+/CD141(BDCA-3)+ myeloid DCs, Ipilimumab, Avelumab	Pembrolizumab, Radiotherapy	NSCLC	Recruiting	II	NCT04571632
Cytokine + Immune checkpoint	IL23 + IL36γ + OX40L (encoded by 3 mRNAs encapsulated in lipid nanoparticles)	mRNA-2752	Durvalumab	HNSCC, Non-hodgkin lymphoma, TNBC, Urothelial cancer, Other solid tumors	Recruiting	I	NCT03739931
Cytokine + nonpathogenic bacterium	BCG + IL2	OncoTICE, Aldesleukin	-	Melanoma	Not yet recruiting	II/III	NCT03928275
Immune checkpoint + PRR	CD40L + CD70 + TLR4 (encoded by 3 naked mRNAs)	TriMix	Chemotherapy, Surgery	Breast cancer	Recruiting	I	NCT03788083
mAb + PRR agonist	Anti-CTLA4 + TLR4 agonist	Ipilimumab, GLA-SE	FOLFOX, Nivolumab	CRC	Withdrawn	I	NCT03982121
	Anti-CTLA4 + TLR9 agonist	Ipilimumab, Tilsotolimod	Nivolumab	Solid tumors	Recruiting	I	NCT04270864
	Anti-PD1 + TLR8 agonist	Nivolumab, Motolimod	-	HNSCC	Recruiting	I	NCT04272333*
	Anti-OX40 + TLR9 agonist	INCAGN01949, CMP-001	-	Pancreatic cancer, Other solid tumors	Not yet recruiting	I/II	NCT04387071
	Anti-OX40 + TLR9 agonist	BMS-986178, SD-101	Radiotherapy	Lymphoma	Recruiting	I	NCT03410901
	Anti-OX40 + TLR9 agonist	BMS-986178, SD-101	BMS-986178	Solid tumors	Recruiting	I	NCT03831295

(*Continued*)

Table 2. (Continued).

Mixed types of *i.t.* immunotherapy							
Types of *i.t.* immunotherapy	Subtypes of *i.t.* immunotherapy	Agents for *i.t.* immunotherapy	Non-*i.t.* co-therapy[*i.t.* co-therapy]	Indications	Trial status	Phases	NCT Number
mAb + Targeted chemotherapy	SUMOylation inhibitor + (Anti-EGFR or Anti-PDL1)	TAK-981, Cetuximab, Avelumab	-	HNSCC	Recruiting	I	NCT04065555*
mAb + PRR agonist	(Anti-CTLA4/Anti-LAG3/NLRP3 agonist) + Anti-PD1	Ipilimumab, Relatlimab, BMS-986299, Nivolumab	Surgery	Solid tumors	Not yet recruiting	I	NCT04541108* (Exp. arm 1&2)
Chemotherapy + PRR agonist	ICD chemoinducers (candidates) ± STING agonist	5-Fluorouracil, Carboplatin, Paclitaxel, TAK-676					

Source: clinicaltrials.gov. Search queries: *Condition or disease*: "cancer" OR "tumor" OR "neoplasm" OR "carcinoma" OR "sarcoma" OR "melanoma" OR "lymphoma" OR "leukemia" OR "myeloma". *Other terms*: "intratumoral" OR "intratumor" OR "intralesional" OR "intralesion" OR "local injection" OR "local administration". *Study starts from* "01/01/2018" *to* "08/06/2021". *Study type*: Interventional. *Phase*: Early Phase 1 to Phase 4. Among the studies that came out from this search, we excluded the ones that were not involving immunotherapy. 153 studies were found to finally concern intratumoral immunotherapies. The subsection "Mixed types of *i.t.* immunotherapy" regroups trials in which various subtypes of immunotherapies are co-injected *i.t.* Of note, when oncolytic viruses, adoptive immune cells, or DC-based vaccines where genetically engineered to express a transgene (e.g. cytokine), they were described in their respective category rather than considered as combination treatments. The clinical trials testing drug administration through the CIVO device are indicated by an asterisk (*). BCG, Bacillus Calmette–Guérin; CAR-T, chimeric antigen receptor T cell; CRC, colorectal cancer; CSCC, cutaneous squamous cell carcinoma; DC, dendritic cell; EDB-FN, extradomain B of fibronectin; FOLFIRINOX, folinic acid+fluorouracil+irinotecan+oxaliplatin; FOLFOX, folinic acid+fluorouracil+oxaliplatin; HNSCC, head and neck squamous cell carcinoma; ICD, immunogenic cell death; ICI, immune checkpoint inhibitor; i.t., intratumor; mAb, monoclonal antibody; Nab, nanoparticle albumin-bound; N/D, not determined; NK, natural killer; NSCLC, non-small-cell lung carcinoma; ODN, oligodeoxynucleotide; PRR, pattern recognition receptor; SCC, squamous cell carcinoma; TNBC, triple negative breast cancer.

at the surface of regulatory $CD4^+$ T lymphocytes (Tregs), which are abundant in the tumor microenvironment (TME), can also trigger Tregs depletion through ADCC, and thus alleviate cancer-associated immunosuppression.[33,104,105]

Clinically approved therapeutic antibodies, including ICIs, are administered systemically and at a high dose that is expected to saturate their target receptors. However, this goal is hardly achieved in the practice following systemic delivery.[106] Moreover, intravenous infusion of ICIs like anti-CTLA4 can trigger dose-dependent (sometimes fatal) immune-related toxic effects. Collectively, these observations are arguing in favor of a local administration. As an illustration, an eight-time reduced dose of anti-CTLA4 injected *i.t.* demonstrated similar antitumor efficacy as systemic injection in mice, but with a leaked dose in the serum 1000 times lower.[107] Along this line, the toxicity of a CD40 agonistic antibody was abolished upon *i.t.* injection.[108] Furthermore, an agonistic antibody binding to glucocorticoid-induced TNF receptor-related protein (GITR, also known as TNFRSF18) showed a superior recruitment and activation of T cells in the TME when injected locally rather than intraperitoneally or *i.v.* in mice.[108,109] Altogether, in addition to efficiently target T cells infiltrating the malignant lesions, *i.t.* injection of therapeutic mAbs may reduce autoimmune and inflammatory side effects, circumvent the unknowns surrounding tumor tissue penetration, and ease the combination of antibodies. Such combinations have been evaluated with success in preclinical studies involving anti-CTLA4 plus anti-OX40, or anti-PD1 plus anti-CD137.[91,110–112] Additionally, some teams are designing a formulation allowing slow *i.t.* release of mAbs for prolonged and improved efficacy.[113]

Along with these preclinical incentives, numerous clinical trials have been launched these recent years to evaluate the therapeutic index of mAbs when administered locally (Table 2). For instance, the Phase I trial NCT04336098 will assess SRF617, an antibody which neutralizes the ecto-nucleotidase CD39 (a catalyzer of the degradation of ATP into immunosuppressive adenosine).[114] SRF617 will be administered locally in patients with advanced solid tumors, either alone or in combination with *i.v.* infusions of either gemcitabine plus albumin-bound paclitaxel, or pembrolizumab. Other antagonistic antibodies which target PD1, PDL1, or CTLA4 are also being tested following *i.t.* delivery, either separately or combined. NCT03889912 will characterize the safety and tolerability of weekly *i.t.* injections of cemiplimab for 12 weeks prior to scheduled surgery in patients with recurrent cutaneous squamous cell carcinoma (CSCC). NCT03316274 will assess *i.t.* administration of nivolumab in participants with Kaposi sarcoma. NCT04090775 will evaluate the efficacy and adverse events related to cryosurgery combined with *i.t* immunotherapy with nivolumab + ipilimumab, together with low-dose cyclophosphamide (injectable and oral) in metastatic prostatic adenocarcinoma. In the Phase II/III trial NCT03755739, transartery/*i.t.* infusion of pembrolizumab and/or ipilimumab will be tested for the treatment of advanced solid tumors. Conversely, several antibodies mediating an agonistic effect on co-stimulatory T lymphocyte receptors have shown promising results in preclinical or clinical settings and are currently investigated as *i.t.* immunotherapy in clinical trials. NCT03792724, a Phase I–II study, will assess *i.t.* urelumab (an agonistic antibody binding to CD137, also known as TNFRSF9 or 4–1BB) combined with systemic nivolumab in patients with advanced solid neoplasms. The trial will recruit 32 participants to measure the incidence of adverse events, determine the recommended dose, and calculate the response rate. NCT03892525 was designed to test *i.t.* selicrelumab (anti-CD40) in combination with *i.v.* atezolizumab (anti-PDL1) in patients with refractory or relapsed B cell lymphoma. However, the study was terminated because drug development stopped. NCT03818542 aimed to evaluate ABBV-927 (anti-CD40) as first-line monotherapy in subjects with locally advanced head and neck squamous cell carcinoma (HNSCC) eligible for surgical resection. Nevertheless, the trial was terminated for strategic considerations. NCT04059588 will evaluate the safety and tolerability of an Fc-engineered anti-CD40 mAb (2141-V11), administered intratumorally for the treatment of patients with metastatic skin lesions. At last, NCT03432741 aimed to determine the feasibility of intralesional microinjections of a broad range of cancer immunotherapies (daratumumab, nivolumab, obinutuzumab, pembrolizumab, rituximab, and trastuzumab) and chemotherapies (belinostat, carfilzomib, gemcitabine, and romidepsin) in patients with lymphoma, or recurrent/resistant stage IV breast cancer. Unfortunately, this pilot Phase I trial is currently suspended for funding reasons (Table 2).

[ii] *Conjugated antibodies*. Immunoconjugates are most frequently constituted of a tumor antigen-targeting antibody covalently paired with a cytotoxic compound. The latter can be a radioactive particle (e.g. isotype yttrium 90 for ibritumomab tiuxetan),[115,116] a toxin (e.g. *Pseudomonas* exotoxin for moxetumomab pasdotox),[117] or a chemotherapeutic agent such as a topoisomerase inhibitor (e.g. SN-38 for sacituzumab govitecan) or a microtubule inhibitor (e.g. auristatin for brentuxumab vedotin, mertansine for trastuzumab emtansine)[118] (Table 1).[119–122] By binding to cancer cells, conjugated antibodies concentrate the cytotoxicants into the tumor bed.[123] In addition to triggering cancer cell death, they promote the release of tumor antigens and danger signals which may elicit an anticancer immune response.[124] For instance, LMB-100 is a humanized anti-mesothelin antibody that is fused with a truncated *Pseudomonas* exotoxin A.[125] The Phase I trial NCT04840615 will evaluate the safety and determine the recommended Phase 2 dose (RP2D) of *i.t.* injections of LMB-100 combined with *i.v.* ipilimumab in patients with pleural or peritoneal mesothelioma. In the same line, NCT04547777 is a dose-escalation study aiming to determine the maximum tolerated dose (MTD) of a single *i.t.* infusion of 2141-V11 (anti-CD40) in combination with the immunotoxin D2C7, delivered *i.t.* at a fixed dose, in patients with recurrent malignant glioma (Table 2). D2C7 is a genetically engineered form of the *Pseudomonas* exotoxin conjugated with a single-chain variable fragment (scFv) harboring a high binding affinity for the epidermal growth factor receptor (EGFR) and its active mutant EGFRvIII; both being overexpressed on glioblastoma cells.

Yet, some immunoconjugates are non-cytotoxic *per se* but promote antitumor immunity. For instance, the Phase I trial NCT04502888 is recruiting patients with CSCC or HNSCC to test *i.t.* delivery of a fusion protein called SL-172154. The latter consists of human signal-regulatory protein alpha and CD40

ligand linked by a Fc fragment (SIRPα-Fc-CD40L).[126] SL-172154 shows dual functionality as it concomitantly antagonizes CD47 on malignant cells and agonizes CD40 on antigen-presenting cells. This dose-escalation study will inform the safety profile of SL-172154, and define the MTD and RP2D (Table 2). Immunocytokines represent another example of non-cytotoxic conjugated antibodies. They will be introduced in the next section of this review.

[iii] *Bispecific antibodies*. Through double antigen affinity, bispecific mAbs bring immune cells in close proximity to tumor cells. For instance, blinatumomab binds to CD19 on leukemia cells and can engage T cells through binding to their CD3 (Table 1).[127] To our knowledge, there were no clinical trials initiated these past 3 y involving intratumoral delivery of bispecific antibodies.

Cytokines

Cytokines are secreted messengers which orchestrate the dynamics and functions of leucocytes. Among them, IL12p70, IFN-I and IFNγ are produced by DCs upon activation and participate in the ignition of T_H1 responses which are critical in cancer immunosurveillance.[128–130] Upon activation, effector T_H1 and T_C1 cells secrete IL2 and IFNγ. Not only these cytokines contribute to sustain the immune response (positive feedback loop), but IFNs also harbor direct cytostatic and pro-apoptotic effects on cancer entities which participate to the overall antineoplastic activity.[131,132] Thus, even though cytokine injection alone is poorly efficient, it enhances adjuvanticity. Aldesleukin,[133] an analog of IL2, the first cytokine to reach the market, showed promising results when injected locally.[76] It is currently the subject of a Phase III clinical trial (NCT03233828) assessing the benefit of two *i.t.* injections of IL2 (as compared to saline in the control group) in suspected or *in situ* melanoma. NCT03982004 evaluated the safety and side effects of intralesional granulocyte-macrophage colony-stimulating factor (GM-CSF)[134] in participants with breast cancer with cutaneous metastases. The cytokine was combined with topical imiquimod, *i.v.* pembrolizumab, and epicutaneous cryoimmunotherapy. However, the trial was terminated by decision of the sponsor. Several analogs of IFNα are also tested for their capacity to activate DCs and NK cells and to slow down the proliferation of cancer cells. They have successfully treated cystic craniopharyngioma in a preliminary clinical study.[135] However, cytokines regulate many biological pathways and their *i.v.* administration provokes important off-target adverse effects.[136,137] For this reason, IL12 immunotherapies have not yet entered Phase III clinical trials due to toxicity.[138,139] Their local administration as such did not notably increase their adjuvanticity and/or reduce toxicity due to a rapid leakage in the systemic circulation.[140] Therefore, to prolong intratumoral retention, the team of Wittrup proposed a formulation in which the cytokine is anchored to a collagen-binding protein, which was successful in mouse models.[138,141] To restrain their expression to targeted tissues, genes encoding cytokines can be inserted into locally injected DNA plasmids, mRNA or viruses (see next sections of this review) allowing cytokines to be recombined *in situ*. This promising strategy is the object of various preclinical and clinical studies (Table 2). For instance, *i.t.* injection of DCs overexpressing IL12 stimulated antitumor immunity and proved efficacy in mouse models.[142,143] Similarly, a Phase 1 trial (NCT03946800) is testing a lipid-encapsulated mRNA encoding IL12 (referred to as MED1191). It will be delivered intratumorally in combination with *i.v.* durvalumab (anti-PDL1) to 87 subjects with advanced solid tumors. The escalation arm will determine the MTD while the expansion arm will measure the overall response rate (ORR). NCT03871348 evaluates *i.t.* administration of SAR441000, a mixture of four mRNAs encoding IL12 single chain, IFNα2b, GM-CSF, and IL15sushi,[144] alone or combined with *i.v.* cemiplimab in advanced solid tumors in a dose-escalation and dose-expansion study.[145] Three Phase II trials study *i.t.* electroporation of tavokinogene telseplasmid (Tavo-EP), a DNA plasmid encoding the human IL12, together with PD1 blockade. In NCT04526730, tavo-EP will be tested as a neoadjuvant treatment combined with systemic infusion of nivolumab in participants with operable locally advanced melanoma. NCT03567720 will combine *i.t.* tavo-EP with *i.v.* pembrolizumab alone or in addition of *i.v.* nab-paclitaxel in triple negative breast cancer (TNBC) patients with cutaneous/subcutaneous neoplasms. At last, NCT03823131 will determine whether *i.t.* tavo-EP combined with *i.v.* pembrolizumab, together or not with epacadostat (inhibitor of indoleamine 2,3-dioxygenase-1 [IDO1]), enhances the best ORR in HNSCC as compared to historical score for pembrolizumab monotherapy.

In order to enhance their bioavailability and improve their tissue targeting, cytokines can be fused with immunoglobulins, thus generating so-called immunocytokines.[76,122] A Phase I/II clinical trial (NCT03958383) will evaluate *i.t.* administration of the immunocytokine hu14.18-IL2, either alone or combined with radiation therapy ± systemic immune checkpoint blockade (nivolumab ± ipilimumab) in patients with advanced or surgically incurable melanoma. On one hand, the hu14.18 moiety binds to the GD2 antigen expressed at the surface of several tumor histotypes (e.g. melanoma and neuroblastoma) while its Fc component mediates ADCC. On the other hand, the IL2 moiety locally stimulates NK and T cell antitumor immune responses.[146] Of note, hu14.18-IL2 has been previously tested clinically (NCT00590824) in patients with resectable recurrent advanced melanoma. This study demonstrated prolonged tumor-free survival following surgery plus 3 courses of hu14.18-IL2.[147,148] NCT04362722, a Phase II trial, investigates the therapeutic potential of single or multiple *i.t.* injection(s) of a cocktail of two immunocytokines, L19IL2 and L19TNF, in patients with basal cell carcinoma or CSCC. These agents consist of a human scFv called L19 which is directed against the extra-domain B of fibronectin (EDB-FN) and fused to either IL2 or TNF. EDB-FN being upregulated in tumor area experiencing neoangiogenesis, L19 contributes to concentrate IL2 in the malignant bed.[149] L19IL2/L19TNF will be administered once weekly for up to 4 weeks into all injectable lesions. Of note, this combination showed promising results in a Phase II clinical trial in patients with advanced metastatic melanoma who were not candidates to surgery (Table 2).[148]

PRR agonists

DCs sense the presence of pathogens by means of their PRRs, such as TLRs located at the plasma membrane or in endosomes, as well as some cytosolic adapters like STING1,[65] melanoma differentiation-associated protein 5 (MDA-5)[150] or retinoic acid-inducible gene I (RIG-I).[151–155] These receptors are also involved in the recognition of DAMPs released by stressed or dying cells.[156,157] Therefore, over the past two decades, their potent immunostimulatory effects have inspired laboratories in the development of PRR agonists as antineoplastic agents in preclinical and clinical settings.[64]

TLR3 agonists

Upon activation by endogenous (mammalian) as well as exogenous (viral) double-stranded ribonucleic acids (dsRNA) in endosomes, TLR3 triggers the secretion of pro-inflammatory cytokines, such as IFN-I, and contributes to DC cross-priming.[79,158] The critical role of IFNs in the initiation of tumor antigen-specific immunity has encouraged the clinical investigation of TLR3 agonists as single agents or in combination with other therapeutics.[22] These agonists have reported immunostimulatory function as well as direct anti-proliferative and pro-apoptotic effects in malignant cells positive for TLR3 (e.g. head and neck carcinoma, lung squamous cell carcinoma and adenocarcinoma).[159] The latter property could extend their application to cancer patients with immunodeficiencies. The prototypical TLR3 agonist polyinosinic-polycytidylic acid (poly-IC) and its derivatives are being evaluated as intratumor interventions in advanced oncological indications.[160] For instance, poly-IC complexed with poly-L-lysine and carboxymethylcellulose (poly-ICLC/Hiltonol) is under investigation in B-cell lymphoma in association with radiotherapy and recombinant human FMS-like tyrosine kinase-3 ligand (Flt3L) (NCT01976585), and in mesothelioma in a neoadjuvant setting (NCT04525859) (Table 2). Already, *i.t.* poly-ICLC has proven well tolerated and safe in advanced cancer patients, as a standalone application as well as in combination therapies, with signs of immunostimulation and some clinical benefits witnessed.[161,162] As another example, BO-112 is a formulation of polyI:C complexed with polyethylenimine. It promotes tumor cell apoptosis with ICD features.[78] In immunocompetent mice bearing ectopic colorectal (MC38, 4T1) and melanoma (B16-F10 tumors), *i.t.* delivery of BO-112 significantly controlled disease progression. This therapeutic effect appeared mediated by IFN-I and IFNγ.[78] Two Phase I trials involving *i.t.* BO-112 have been initiated these past 3 y. NCT04420975 is primarily evaluating the frequency and severity of adverse events and determining dose-limiting toxicities (DLT) of BO-112 when combined with *i.v.* nivolumab plus standard of care radiotherapy before surgical resection in patients with soft tissue sarcoma (Table 2). NCT04777708 will assess the clinical response to local delivery of BO-112 together with *i.v.* pembrolizumab in patients with hepatocellular carcinoma (Table 2).

TLR4 agonists

Despite the numerous TLR4 ligands that have been the subject of preclinical and clinical investigations, only two have been approved by the FDA for clinical use as cancer treatment: BCG and monophosphoryl lipid A (MPL).[163,164] NCT03742804 aimed to test G100 as single agent in lymphoma but has been withdrawn because the study sponsor sold and the new owner did not support the study.

TLR7 agonists

TLR7 is an intracellular receptor expressed on endosomal membranes which recognizes nucleosides and nucleotides from intracellular pathogens.[165] Upon activation, TLR7 can induce an inflammatory response and the production of IFN-I, both beneficial for triggering antitumor immunity.[63,166] In this line, imiquimod received FDA approval as topical standalone application (cream) to activate TLR7 in the environment of superficial basal cell carcinoma. The Phase I NCT03301896 consists of four dose-escalation and two dose-expansion parts testing the TLR7 agonist LHC165 given *i.t.* as monotherapy or in combination with *i.v.* PDR001 (anti-PD1) for the treatment of solid tumors (Table 2). Similarly, NCT04799054 will evaluate the TLR7/8 agonist TransCon as a single agent or combined with *i.v.* pembrolizumab in dose-escalation and dose-expansion scenarios for patients with advanced or metastatic solid tumors (Table 2). At last, NCT03435640 will assess *i.t.* delivery of the TLR7/8 agonist NKTR-262 in 3-week treatment cycles with systemic administration of bempegaldesleukin, together or not with nivolumab, in patients with locally advanced or metastatic solid tumors (Table 2). Of note, bempegaldesleukin consists of a pegylated IL2 which binds to the β subunit of the IL2 receptor, thus preferentially expanding $CD8^+$ T cells over immunosuppressive regulatory $CD4^+$ T cells.[167,168]

TLR8 agonists

Similarly to TLR7, detection of single-stranded (ss)RNA by TLR8 induces an inflammatory response and IFN-I secretion.[169] NCT03906526 studies the safety and tolerability profile of *i.t.* and *s.c.* delivery of the TLR8 agonist motolimod (VTX-2337) in combination with *i.v.* nivolumab in patients with HNSCC. Meanwhile, tumor immune modulation will be evaluated by quantitating $CD8^+$ T cells infiltrating the neoplastic microenvironment before and after treatment (Table 2).

TRL9 agonists

TLR9 is predominantly located intracellularly in immune cells, such as antigen-presenting cells (e.g. conventional and plasmacytoid DCs, macrophages, B cells) or T lymphocytes.[170] The main ligands of TLR9 are bacterial unmethylated cytidine phosphate guanosine (CpG) oligodeoxynucleotides (ODNs). Stimulation of TLR9 triggers the production of pro-inflammatory cytokines (e.g. IFN-I, IL6, IL12, TNFα) which activates innate immune actors, including DC and NK cells. In turn, antigen cross-presentation by mature DCs primes the adaptive arm of the immune system which culminates in the destruction of microorganisms or cancer cells.[171–173] For the purpose of cancer treatment, unmethylated CpG ODNs have been synthesized to mimic the immunostimulatory activity of bacterial DNA on TLR9.[174] Intratumoral delivery of the TLR9

agonist cavrotolimod (AST-008) is being evaluated in a Phase Ib/II trial (NCT03684785) as a standalone or in combination with infusions of either cemiplimab or pembrolizumab in skin cancers and solid tumors with liver metastases (Table 2). CMP-001 is a CpG ODN packaged in virus-like particles. CMP-001 is being tested following *i.t.* delivery in combination with infusions of ICIs targeting CTLA4 (ipilimumab), PD1 (cemiplimab, nivolumab, or pembrolizumab) or PDL1 (atezolizumab), together or not with radiotherapy or surgery, in multiple oncological indications (Table 2). NCT03983668 will determine the DLT of *i.t.* CMP-001 in combination with *i.v.* pembrolizumab in patients with relapsed and refractory lymphomas. The efficacy of this combination treatment will be evaluated in Phase II trials in advanced operable melanoma (NCT04708418) and in HNSCC participants who have not been previously treated with PD1 blockers (NCT04633278). Four Phase II (or II/III) trials will evaluate *i.v.* nivolumab associated with intratumor CMP-001 in melanoma. NCT04698187 and NCT03618641 will measure the response rate to the latter combinatorial therapy as well as survival of subjects with refractory unresectable/metastatic melanoma or Stage III melanoma with clinically apparent lymph node involvement, respectively. NCT04401995 and NCT04695977 will compare nivolumab monotherapy to nivolumab plus *i.t.* CMP-001 in a neoadjuvant setting in patients with Stage III melanoma, or as a first line treatment in participants with unresectable or metastatic melanoma, respectively. NCT04916002 will determine the safety, tolerability and efficacy of i.t. CMP-001 in combination with cemiplimab in patients with advanced/metastatic CSCC, Merkel cell carcinoma (MCC), or TNBC. Three clinical trials will investigate CMP-001 in association with radiotherapy. NCT03507699 aims to test a combinatorial regimen of *s.c.* CMP-001, *i.v.* nivolumab, *i.v.* ipilimumab, and 21-gray (Gy) liver radiation therapy in patients with colorectal cancer (CRC) with liver metastases. NCT04807192 will assess preoperative stereotactic body radiation therapy (SBRT) alone or combined with *s.c.* followed by *i.t.* administrations of CMP-001 in patients with early stage TNBC. The completed Phase I study NCT03438318 has evaluated i) the safety and efficacy of *i.t.* CMP-001 together with atezolizumab, and ii) the benefit of radiation therapy to this combination. The synthetic TLR9 agonist (also known as IMO-2125) in evaluated in Phase I–III trials in co-treatment with infused immune checkpoint antagonists and agonists. More precisely, the Phase I trial NCT04196283 assesses *i.t.* tilsotolimod plus *i.v.* ABBV-368 (anti-OX40) in combination with *i.v.* nab-paclitaxel alone or combined with *i.v.* ABBV-181 (anti-PD1) in patients with recurrent/metastatic HNSCC. The Phase II study NCT03865082 intends to test the efficacy of *i.t.* tilsotolimod with ipilimumab and nivolumab in different solid tumors. The Phase III trial NCT03445533 will study the benefits of *i.t.* tilsotolimod and *i.v.* ipilimumab in advanced melanoma. Of note, a previous Phase I/II trial assessing *i.t.* tilsotolimod with systemic ipilimumab in patients with anti-PD1-resistant advanced melanoma showed a rapid induction of local IFNα gene signature, DC maturation and antigen presentation, as well as an expansion of specific T cells; all of which correlated with clinical response.[175] The synthetic CpG ODN SD-101 is being investigated together with radiotherapy in various oncological indications. NCT03007732 (Phase II) evaluates *i.t.* SD-101 with oral prednisone (corticosteroids) and abiraterone acetate (androgen synthesis inhibitor), *i.m.* leuprolide acetate (gonadotropin-releasing hormone receptor [GnRHR] agonist), SBRT, and *i.v.* pembrolizumab in patients with newly diagnosed hormone-naive oligometastatic prostate cancer. NCT03322384 (Phase I/II) assesses the efficacy of five intralesional injections of SD101, radiotherapy and oral epacadostat for advanced/refractory solid tumors and lymphoma. NCT04050085 studies the side effects of *i.t.* SD-101 when delivered together with *i.v.* nivolumab and radiation therapy in patients with chemotherapy-refractory and metastatic pancreatic cancer. At last, NCT04612530 will evaluate the safety and efficacy of irreversible electroporation (IRE) + nivolumab + *i.t.* CpG ODN in pancreatic cancer patients, as compared to IRE + nivolumab, and nivolumab monotherapy. Of note, IRE is a recent technique relying on electrical pulses which allows local ablation of malignant lesions. Its cytoreductive propensity is accompanied with antigen release and stimulation of a T cell immune response, thus encouraging its combination with immunotherapy.[176]

STING agonists

In the tumoral context, upon detection of accumulated mitochondria- or nucleus-derived dsDNA leaking in the cytosol of cells treated by chemotherapy or radiotherapy, STING induces the production of IFN-I which are critical for the initiation of antitumor immune responses. Therefore, the pharmaceutical industry has generated direct STING activators for oncological indications tested either alone or with various chemotherapeutic and immunotherapeutic combinatorial regimens.[67,177,178] In this dynamic, several Phase I–II trials have been recently initiated to investigate *i.t.* delivery of the STING agonists ADU-S100, BI 1387446, E7766, IMSA101, MK-1454, and SYNB1891 (Table 2). The Phase I trials NCT04144140 aims to assess safety, tolerability, and preliminary clinical activity of *i.t.* E7766 as a standalone agent in patients with advanced solid tumors or lymphomas. NCT04167137 will determine single-agent MTD of *i.t.* SYNB1891 as monotherapy, and the RP2D in combination with atezolizumab, in subjects diagnosed with advanced/metastatic solid tumors and lymphoma. The Phase II NCT03937141 evaluates the ORR to *i.t.* ADU-S100 combined with *i.v.* pembrolizumab as first-line treatment of adults with PDL1-positive recurrent or metastatic HNSCC. Similarly, NCT04220866 studies *i.t.* MK-1454 in combination with *i.v.* pembrolizumab, compared to pembrolizumab alone, as a first-intention treatment of subjects with metastatic, or unresectable, recurrent HNSCC. NCT04020185 is a dose-escalation (Phase I) and dose-expansion (Phase IIA) study of participants receiving *i.t.* IMSA101 alone or combined with ICI. The Phase I NCT04147234 will assess *i.t.* BI 1387446 in adults with advanced cancer that failed previous treatment.[179] In addition, some participants will receive *i.v.* infusions of ezabenlimab (formerly BI 754091), a humanized IgG4 anti-PD1 mAb, every 3 weeks.

RIG-I agonists

RIG-I-like receptors are key sensors of virus infection as well as host-derived RNA. Their detection induces the transcription of immune genes including IFN-I. As a consequence, synthetic

RIG-I agonists have been synthesized and evaluated in preclinical and clinical studies as vaccine adjuvants, or potentiators of anticancer immunotherapies.[151,180–182] For instance, the completed Phase I NCT03739138 aimed to assess safety, tolerability, pharmacokinetics, and preliminary antitumor activity of *i.t.* injections of MK-4621 delivered via a nucleic acid delivery system, JetPEI™, as monotherapy, and in combination with pembrolizumab in patients with advanced/metastatic solid tumors with no available results yet (Table 2).

Nonpathogenic bacteria

Since the early approval of BCG in bladder cancer, additional weakened strains of bacteria are applying to join the armamentarium of cancer treatments. By stimulating multiple PRRs, such as TLR2 and TLR4, and by providing xenoantigens, bacteria attract and activate immune sentinels, and can stimulate antitumor immune activity with reduced toxicity.[183–185] In particular, anaerobic strains, which preferentially replicate within tumors, are the objects of several clinical trials. For instance, *Clostridium novyi*-NT[186–188] is being tested *i.t.* in combination with *i.v.* pembrolizumab and oral doxycycline in subjects with advanced solid malignancies (NCT03435952) (Table 2). While patient enrollment continues, intermediary results from nine patients demonstrated encouraging signals of antineoplastic activity and a manageable toxicity profile.[189]

Biopolymers

On top of antibodies, cytokines, and PRR agonists, some biopolymers can be endowed with adjuvant properties. Two of them, copaxone (glatiramer acetate) and IP-001 (N-dihydrogalactochitosan), are evaluated clinically as intratumoral cancer immunotherapy (Table 2). As such, copaxone is being administered prior to standard of care surgery in patients with percutaneously accessible tumors (NCT03982212). IP-001 is evaluated in a Phase I/II trial following thermal ablation in advanced solid tumors (NCT03993678).

ICD-inducing synthetic agents

In 2005, it was shown that anthracycline-based chemotherapy triggers an immunogenic, rather than tolerogenic, cancer cell apoptosis. Dubbed as immunogenic cell death or ICD, this phenomenon is characterized by the exposure/release of DAMPs along with the spread of antigens that are captured by immature DCs, thus promoting an adaptive immune response.[15,190,191] Since this first report on ICD, multiple cancer treatment modalities have been reported to be endowed with such propensity.[192,193] They include additional chemotherapeutics (e.g. cyclophosphamide and oxaliplatin), certain physical cues (e.g. radiotherapy and photodynamic therapy), oncolytic viruses (e.g. T-VEC), and some lytic peptides (e.g. LTX-315).[16,69,194–197] An updated list of all confirmed ICD-inducing agents can be found in the following review:[16] Candidates for ICD induction are also indexed such as the platinum salts cisplatin and carboplatin, gemcitabine, pemetrexed, vinca-alkaloids, or taxanes, whose cell death can be accompanied by the release of ICD-related DAMPs, at least in certain cell lines.[16] Of note, with the exception of the oncolytic virus T-VEC, ICD-inducing interventions were not reported in Table 1 because they were not approved for their immune stimulating potential and are thus not commonly classified as immunotherapeutics.

Interestingly, two trials are evaluating *i.t.* cisplatin, either as a single agent in non-small-cell lung carcinoma (NSCLC) (NCT04809103), or in a formulation referred to as INT230-6 (in which it is combined with vinblastine[198] and the excipient salcaprozate sodium[199]) in breast cancer (NCT04781725) (Table 2). NCT04701645 is a pilot study that will assess the feasibility of implanting in selected tumor deposits some innovative microdevices which diffuse up to 20 drugs locally. Patients with resectable lesions of ovarian, fallopian tube, and peritoneal cancers will be recruited to this purpose. Clinically relevant drugs delivered (alone or in combination) will include some confirmed or potential ICD inducers: carboplatin, cisplatin, cyclophosphamide, doxorubicin, gemcitabine, paclitaxel, pemetrexed, and/or vinorelbine. Appearance of adverse events and response to treatments will be measured (Table 2).

Intralesional administration of ICD-inducing peptides like CyPep-1 and LTX-315 has proven antitumor activity in preclinical studies.[194,200–202] These data have motivated their translation into the clinic with two Phase II trials recently engaged. First, NCT04796194 aims to assess the ORR of intratumoral LTX-315, a peptide derived from human lactoferrin, in combination with systemic PD1 blockade in patients with percutaneously accessible advanced solid tumors. Second, NCT04260529 is a dose-escalation study that will assess the safety and tolerability of the synthetic oncolytic peptide CyPep-1 and determine its RP2D in advanced solid malignancies (Table 2).

Oncolytic virotherapy

OVs selectively infect and kill tumor cells in an immunogenic fashion. Indeed, viral oncolysis releases in the extracellular space both tumor and viral antigens together with danger signals, thus triggering the recruitment of DCs. Numerous OVs are administered locally and have the advantage to prime a polyclonal immune response without requiring tumor antigen identification and administration as a transgene, or as a tumor antigen provided either naked or loaded in DCs.[68,203,204] A myriad of OVs are genetically engineered to express immunomodulatory proteins (e.g. cytokines) in order to boost the activation of the immune system.[70,205] For more information on oncolytic viruses, a series of Trial Watch dedicated to the topic has been published.[68,206,207]

Herpesviruses

Some oncolytic viruses have been armed with a transgene expressing GM-CSF in order to enhance antitumor immune responses.[70,208–210] This strategy has been applied to the type 1 herpes simplex virus (HSV) T-VEC approved for intratumoral virotherapy of melanoma (Table 2).[70,211,212] T-VEC is investigated as a single agent in recent trials such as NCT03458117 in locally advanced non-melanoma skin cancer, NCT03921073 in

cutaneous angiosarcoma, and NCT04065152 in Kaposi sarcoma. T-VEC is given as a neoadjuvant, either as a standalone in high-risk early melanoma (NCT04427306), or combined with nivolumab in early metastatic melanoma (NCT04330430), or with *i.v.* pembrolizumab prior to complete lymph node dissection in resectable Stage 3 cutaneous melanoma (NCT03842943). NCT04068181 is a Phase II trial aiming at testing T-VEC in melanoma in combination with *i.v.* pembrolizumab following disease progression on prior PD1 blockade or as an adjuvant to PD1 therapy. Other Phase I trials combine T-VEC with *i.v.* atezolizumab: NCT03802604 in operable early breast cancer with residual disease after neoadjuvant chemotherapy, and NCT03256344 in triple negative breast cancer and CRC with liver metastases. NCT04599062 will test T-VEC combined with external beam radiation therapy in soft tissue sarcoma.

Moreover, other oncolytic HSVs equipped with a GM-CSF transgene are tested *i.t.* in clinical trials (Table 2): RP1[213] either as a single agent (NCT04349436) or with the anti-PD1 cemiplimab in advanced CSCC (NCT04050436), OrienX010 in melanoma patients in association with anti-PD1, either toripalimab (NCT04197882) or JSS001 (NCT04206358),[214] or else the HSV-2 OH2 in combination with *i.v.* LP002 (anti-PD1) in cancers of the digestive system (NCT04755543).[215]

At last, additional strains of HSV-1 are clinically evaluated following *i.t.* delivery: i) G207 with radiation therapy in recurrent/progressive pediatric high-grade glioma (NCT04482933),[216,217] ii) RP3 (which expresses proprietary stimulatory agents)[218] combined with anti-PD1 in advanced solid tumors (NCT04735978), iii) ONCR-177 (which encodes CCL4, IL-12, Flt3L, anti-CTLA4, anti-PD1) alone or combined with pembrolizumab in advanced/metastatic solid tumors (NCT04348916),[219] or iv) VG161 (which expresses IL12, IL15 with its receptor alpha unit, and a PDL1 blocking peptide)[218] as single agent in solid tumors including liver cancer (NCT04758897, NCT04806464).

Adenoviruses

With 15 ongoing studies initiated since 2018, adenovirus is the second most common OV in clinical trials and have been among the earliest OVs to enter clinical examination.[220–222] Two recent trials are evaluating adenoviruses as single agent (Table 2). The dose-escalating Phase I/II trial NCT04097002 will test ORCA-010 as first-line therapy against localized prostate adenocarcinoma.[221,223] NCT04673942 is a dose-escalation study of AdAPT-001, expressing TGFβ, in subjects with refractory solid tumors. Oncolytic adenoviruses are also combined with other treatments in the clinic (Table 2).[224] NCT04714983 evaluates DNX-2440 (expressing OX40L)[225] in patients with resectable multifocal liver metastasis scheduled for curative-intent liver resection surgery. NCT03225989 studies LOAd703 (encoding a trimerized membrane-bound extracellular CD40L and 4–1BBL)[226] in colorectal, biliary, ovarian, or pancreatic cancer together with gemcitabine immune-conditioning or standard of care chemotherapy. The Phase II trial NCT04685499 evaluates *i.t.* telomelysin with *i.v.* pembrolizumab in either recurrent inoperable or progressive head and neck squamous cell carcinoma (HNSCC).[227] NCT04391049 studies the side effects of *i.t.* telomelysin when given together with *i.v.* carboplatin, *i.v.* paclitaxel, and radiation therapy.[228] NCT03514836 aimed to evaluate the combination of the oncolytic adenovirus ONCOS-102 (armed with GM-CSF)[206] delivered *i.t.*, together with *s.c.* administration of the DC-based prostate cancer vaccine stapuldencel T (also known as DCVac/PCa) and *i.v.* cyclophosphamide in patients with castration-resistant advanced metastatic prostate cancer. Unfortunately, this trial was terminated due to insufficient accrual. NCT03740256 is a Phase I trial studying the incidence of DLT in patients receiving *i.t.* CAdVEC in combination with adoptively transferred HER2-specific CAR-T cells in participants with $HER2^{+}$ cancer.[66]

Poxviruses

In 1796, Edward Jenner pioneered the concept of vaccines by inoculating attenuated strains of vaccinia virus to vaccinate humans against variola, the agent causing smallpox.[229] In 1840, it became the world's first vaccine and soon the more extensively used for human immunization as well as the more effective public health intervention in human history.[230] Since the disease eradication in the late 1970s, even though national vaccination programs ended, continuous research on vaccinia virus has produced numerous strains with improved safety profiles, some of them with oncolytic activity.[231] Recently initiated trials are evaluating five oncolytic strains of vaccinia virus either as single agents or in combination with ICIs (Table 2). The Phase I NCT03954067 aims to evaluate the safety and tolerability of ASP9801 (which expresses IL7 and IL12) as a single agent and determine the RP2D in (sub)cutaneous and visceral tumors. The dose-escalation trial NCT04725331 evaluates repeated *i.t.* injections of BT-001, alone or combined with *i.v.* infusions of pembrolizumab, in skin cancers, NSCLC, sarcoma, or TNBC. Of note, BT-001 is equipped with transgenes encoding a Treg-depleting anti-CTLA4 and GM-CSF.[232] Similarly, NCT04787003 aims to define the MTD of *i.t.* OVV-01, and evaluate its efficacy with or without pembrolizumab or atezolizumab in patients with advanced malignancies. Likewise, NCT04301011 determines the RP2D of *i.t* Tbio-6517 (which encodes anti-CTLA-4 mAb, Flt3L and IL12), alone and combined to *i.v.* infusions of pembrolizumab in patients with solid tumors. Pexastimogene devacirepvec (Pexa-Vec, previously known as JX-594)[233] is based on the Wyeth strain of vaccinia virus. Pexa-Vec has been genetically inactivated for the gene encoding the viral thymidine kinase, and engineered to express the human GM-CSF and β-galactosidase. NCT03294083 will determine the safety and efficacy of *i.t. versus i.v.* Pexa-Vec combined with *i.v.* cemiplimab in patients with advanced renal cell carcinoma. Preliminary results supported that participants may benefit from *i.v.* Pexa-Vec + cemiplimab with an acceptable safety profile while further investigation is ongoing regarding *i.t.* Pexa-Vec + cemiplimab.[234] Finally, NCT04849260 will first determine the RP2D of Pexa-Vec combined with the anti-PDL1 antibody

ZKAB001 in patients with local progression of failed first-line treatment or metastatic melanoma. In the Phase II of the trial, ORR and progression-free survival (PFS) will be estimated.

Paramyxoviruses

Attenuated measle viruses (MV) are studied in two clinical trials (Table 2). MV-s-NAP,[235] which encodes a secretory form of *H. pylori* neutrophil-activating protein (s-NAP), is evaluated as a single agent in invasive metastatic breast cancer (NCT04521764). Additionally, NCT04195373 aimed to test the safety and tolerability of TMV-018, an oncolytic measle virus encoding the prodrug converting enzyme "super cytosine deaminase", in patients with gastrointestinal tumors. The trial was designed to evaluate TMV-018 either alone and in combination with either 5-fluorocytosine or an anti-PD1, or both. However, no subjects could be recruited leading to the assay withdrawal.

Rhabdoviruses

The Phase I trial NCT03865212 studies an oncolytic vesicular stomatitis *virus engineered to express human IFNβ and the melanocyte lineage-specific antigen* tyrosinase-related protein 1 (VSV-IFNbetaTYRP1) (Table 2). The latter transgene intends to induce an immune response specific to melanoma cells while the former contributes to further stimulate immune actors.[236–240] The MTD of VSV-IFNbetaTYRP1 will be determined following *i.t.* plus *i.v.* delivery in patients with advanced melanoma. By contrast, Voyager V1 (VV1) is a VSV expressing human IFNβ and the human sodium iodide symporter (NIS) for virus tracking by tomography.[241] The Phase II trial NCT04291105 will measure anti-tumor activity of VV1 given both *i.t.* and *i.v.* in combination with *i.v.* cemiplimab in melanoma (Table 2). In the Phase I NCT03618953, an oncolytic MG1 Maraba virus expressing the HPV E6 and E7 antigens (referred to as MG1-E6E7)[242] will be administered *i.v.* (Arm 1) as well as *i.t.* and *i.v.* (Arm 2) following *i.m.* injection of an adenovirus vaccine expressing the same antigens (Ad-E6E7) (Table 2). This heterologous prime-boost oncolytic vaccination will be followed by a systemic infusion of atezolizumab. Similarly, NCT03773744 was a dose-escalation trial of MG1-MAGEA3 given in a heterologous prime-boost vaccination with Ad-MAGEA3[243] in patients with previously treated metastatic melanoma or CSCC. Following Ad-MAGEA3 vaccine priming and pembrolizumab infusion, MG1-MAGEA3 vaccine booster should have been administered first *i.v.* then repeatedly by *i.t.* injections. Unfortunately, NCT03773744 had to be withdrawn due to insufficient drug supply (Table 2).

Picornaviruses

Two recent clinical trials aim to assess safety, tolerability, and efficacy of intratumoral viroimmunotherapy with the coxsackievirus A21, referred to as V937 (formerly CAVATAK®) (Table 2). It will be combined with systemic infusion of pembrolizumab in ICI-naïve participants with advanced/metastatic melanoma (NCT04152863) or in cohorts of patients with advanced TNBC, SCC, HCC, gastric cancer, or solid tumors with liver metastases (NCT04521621). Four clinical trials are evaluating PVSRIPO, a modified live attenuated non-neurovirulent poliovirus which ignites an antitumoral immunity following tumor cell oncolysis (Table 2). Its natural oncotropism relies on the poliovirus receptor PVR/CD155, an oncofetal antigen overexpressed on tumor cells and used for cell entry.[244,245] Preliminary results from the Phase I trial NCT03712358 showed that *i.t.* administration of PVSRIPO was well tolerated and demonstrated promising antitumor activity in patients with advanced melanoma.[246] PVSRIPO will also be tested in combination with *i.v.* nivolumab in the same oncological indication in patients that failed responding to prior PD-1 blockade (NCT04125719, withdrawn with a resubmission planned). The Phase 2 single-arm trial NCT04479241 will evaluate the safety, tolerability, and initial efficacy of *i.t.* PVSRIPO followed by *i.v* pembrolizumab in patients with recurrent brain tumors.[247] At last, the early Phase I trial NCT03564782 is recruiting participants to study PVSRIPO bioactivity in the tumor bed after *i.t.* injection in women with invasive breast cancer scheduled for surgery.

Other virus-based immunotherapies

Non-oncolytic viruses are also applied for cancer immunotherapy as they are sufficient to render tumors more immunogenic by providing both DAMPs and foreign antigens. Moreover, they can be armed with transgenes which further stimulate antitumor pathways (e.g. cytokines and tumor antigens), or inactivate protumor signaling (e.g. tumor suppressors).[248,249]

Adenoviruses

Three studies evaluate the safety and tolerability of Ad-RTS-hIL12 in glioblastoma patients (Table 2). This adenoviral vector allows conditional expression of human IL12 inducible by administration of the small-molecule veledimex. Ad-RTS-hIL12 is administered once *i.t.* upon standard of care craniotomy and tumor resection. Patients are given oral veledimex prior to surgery and for the following 14 d (NCT03679754, Phase I). Of note, a previous Phase I trial (NCT02026271) showed that this veledimex-regulated transcriptional switch safely controls the dose of IL12 delivered and was well tolerated in glioma patients.[139] Ad-RTS-hIL12 plus veledimex is also tested in combination with infusions of anti-PD1: nivolumab (NCT03636477, Phase I) or cemiplimab-rwlc (NCT04006119, Phase II). Other replication-incompetent adenoviruses are considered for cancer immunotherapy together with ICIs (Table 2). NCT04013334 will test *i.t.* injections of the virus MTG201 in combination with 4 weekly *i.v.* infusions of nivolumab in patients with relapsed malignant pleural mesothelioma. MTG201 is equipped with a transgene encoding the tumor suppressor DKK3/REIC (Dickkopf-3/Reduced expression in immortalized cells) whose expression in cancer cells leads to apoptosis, while overexpression in stromal cells of the TME (e.g. fibroblasts) stimulates antitumor immunity.[250] In preclinical studies, a *p53*-armed adenoviral vector (Ad-p53), alone or combined with PD1 blockade, showed an enhanced control of local treated and distant untreated tumors compared

with anti-PD1 alone, suggesting that Ad-p53 immunotherapy mediates abscopal effects and can reverse resistance to ICI.[249,251,252] These results encouraged the clinical evaluation of Ad-p53 in combination with PD1 blockade. Along this line, the Phase I–II investigation NCT02842125 aimed at evaluating the safety and efficacy of *i.t.* Ad-*p53* in combination with *i.v.* nivolumab in patients with recurrent HNSCC. However, this trial was terminated as the cohort has been rolled into a parallel study. In the Phase II trial NCT03544723, *i.t.* Ad-*p53* will be experimented in participants with recurrent or metastatic solid tumors together with *i.v.* infusions of physician's choice of approved anti-PD1/anti-PDL1.

Poxvirus

A total of 22 patients with localized prostate cancer were scheduled to be enrolled in NCT04020094 to receive pre-operative *i.t.* injections of MVA-BN-Brachyury. This latter is a modified vaccinia Ankara-Bavarian Nordic expressing a MHC-I-restricted epitope of the transcription factor Brachyury and three co-stimulatory molecules: B7.1, ICAM-1 and LFA-3. MVA-BN-Brachyury was accompanied with both neoadjuvant and adjuvant combinations of atezolizumab (infusion) plus PROSTVAC (*s.c.*); a vaccinia virus-based vaccine encoding prostate-specific antigen (PSA).[253] The study aimed to assess the safety of this combination immunotherapy and quantify tumor-infiltrating CTLs.[254] However, it was stopped due to funding withdrawal (Table 2).

Arenaviruses

For treating human papilloma virus (HPV)-related cancers, HB-201, an attenuated lymphocytic choriomeningitis virus (LCMV) encoding an inactivated fusion protein constituted of the HPV-16 oncoproteins E6 and E7, is being tested *i.t.* in HNSCC and cervical cancers.[248] HB-201 will be injected once either as a standalone treatment or combined with chemoradiation in the Phase I trial NCT04630353. By contrast, HB-201 will be integrated in a prime-boost immunization setting, in which vaccine recalls are perpetuated with systemic infusions of either HB-201 or the Pichinde virus-based E6/E7 vaccine (HB-202) in the Phase I/II study NCT04180215 (Table 2).

Flavivirus

The Phase I NCT03990493 evaluates *i.t.* injection of the attenuated strain #45AZ5 of dengue virus-1 (referred to as PV-001-DV), combined with *i.v.* infusions of autologous monocyte-derived DCs pulsed with tumor lysate (PV-001-DC), in advanced melanoma (Table 2).[255]

Orthomyxoviruses

Influenza vaccines will be investigated as local therapy of oncological indications in two recently registered trials (Table 2).[256] The explorative Phase II trial NCT04591379 will determine the safety and efficacy of *i.t.* influenza vaccine as an immune response-enhancing treatment before intended curative surgery in participants with CRC. NCT04697576 investigates influenza vaccine delivered first *i.m.* then *i.t.*, either prior to surgery in patients with resectable melanoma, or concurrent with standard of care ipilimumab, nivolumab, or pembrolizumab in metastatic melanoma.

Therapeutic vaccines

Therapeutic cancer vaccines are designed to prime an adaptive immunity against neoplastic cells.[257,258] They rely on providing an abundant source of tumor-specific antigens (TSAs) or tumor-associated antigens (TAAs) in order to favor their capture and (cross-) presentation to T cells by endogenous DCs. This strategy has the advantage to stimulate a cellular immune response that is highly specific to malignant entities. Cancer vaccines exist in different forms: i) cell lysates, ii) purified antigens, iii) recombinant DNA, RNA, or viruses encoding antigenic determinants, or iv) DCs presenting antigens.[259–260] In this dynamic, some efforts are being made for the design of bioinformatics algorithms predicting the binding of antigen epitopes to major histocompatibility complex molecules.[261] A proteogenomic approach combining mass spectrometry and RNA sequencing has also been developed for the identification of tumor antigens, including aberrantly expressed TSAs, which remained undetected with previous methods.[262,263]

Intralesional delivery of cancer vaccines is currently the object of several preclinical[264,265] as well as clinical investigations. In this line, two studies are evaluating the safety of intratumor Ifx-Hu2.0 applied for the care of skin cancers. Ifx-Hu2.0 is a DNA plasmid encoding the highly immunogenic Emm55 streptococcal antigen. It will be administered repeatedly in up to three lesions of cutaneous, subcutaneous or nodal melanoma (NCT03655756) or of MCC or SCC (NCT04160065) (Table 2).[266,267] Recombinant viruses are also tested in patients for the local production of large amounts of tumor antigens, such as HB-201 and MG1-E6E7 in HPV-related cancers, or MVA-BN-Brachyury in prostate cancer (see previous sections of this review) (Table 2). Since the approval of sipuleucel-T,[268,269] cancer vaccine candidates relying on DCs loaded with tumor antigens are expanding (e.g. the aforementioned DCVac/Pca and PV-001-DC). To present antigens and/or express immune cell-activating factors, autologous DCs are extracted from patients, then either genetically manipulated or co-cultured with a tumor lysate or antigens.[269,270] Some DC-based vaccines are being evaluated clinically following *i.t.* delivery. First, the Phase I trial NCT03638765 will take advantage of an intraventricular catheter system (Ommaya)[271] to deliver activated unloaded autologous DCs (DCVax-Direct) to brain metastases of breast or lung primary tumors (Table 2). The main challenge with *i.t.* injection of DCs is to overcome the highly immunosuppressive intratumoral microenvironment which may impair their function. For this reason, some groups have genetically manipulated DCs to overproduce inflammatory cytokines or co-administered them with adjuvants. For instance, Ad-CCL21-DC is a cancer vaccine comprised of autologous DCs transduced *ex vivo* with an adenovirus containing the CCL21 gene, a chemokine which drives DC migration and harbors antineoplastic activities.[272] This latter approach has been reported to induce efficient anticancer immunity in mouse models of leukemia.[273] Ad-CCL21-DC is currently

evaluated following *i.t.* delivery in combination with *i.v.* pembrolizumab for the treatment of NSCLC (NCT03546361) (Table 2).[274]

Adoptive transfer of lymphocytes

Adoptive cell transfer consists in taking the patient's own immune cells (e.g. T cells, NK cells, and DCs), expanding and eventually modifying them *ex vivo*, before reinjecting them to the patient where they will promote or elicit cytotoxic action on cancer cells.[95,275] The low count of cells reaching the lesions after systemic injection may result in suboptimal efficacy. To circumvent this limitation, *i.t.* injection enables to reach higher concentration of effector cells *in situ*, and to raise the chance of overcoming immunosuppression. On top of the adoptive transfer of DCs previously evoked, other strategies consist in expanding *ex vivo* autologous NK cells or tumor-infiltrating T lymphocytes in order to deliver a high dose of cytotoxic cells into the lesion.[276,277] Two clinical trials are using *ex vivo* expanded autologous NK cells as single agent in patients with high grade (NCT04254419) or recurrent glioma (NCT04489420) using an intraventricular catheter system (Table 2).[271] However, not all patients develop tumor-specific T cells. Chimeric antigen receptor T cell (CAR-T) therapy has been thought to overcome this burden. It consists of autologous T cells which are extracted from patients and genetically modified to harbor a chimeric receptor consisting of an scFv targeting a surface tumor antigen. Four CD19 CAR-T cell therapies, namely axicabtagene ciloleucel (Yescarta™), brexucabtagene autoleucel (Tecartus™), lisocabtagene maraleucel (Breyanzi™), and tisagenlecleucel (Kymriah™) are currently approved as a systemic intervention in leukemia/lymphoma-bearing patients.[278,279] A fifth CAR-T cell targeting B cell maturation antigen (BCMA) also received FDA approval in multiple myeloma in March 2021 (Table 1).[280–283] The Phase I trial NCT03389230 is recruiting patients with recurrent or refractory Grade II–IV glioma to investigate *i.t.* delivery of memory-enriched T cells lentivirally transduced to express a human epidermal growth factor receptor 2 (HER2)-specific, hinge-optimized, 41BB-costimulatory chimeric receptor and a truncated CD19 (HER2(EQ)BBζ/CD19t+). The study will measure the incidence of severe adverse events and determine RP2D, describe persistence and expansion of the CAR-T cells, and estimate median OS. NCT03932565 evaluates a fourth generation CAR-T cells targeting Nectin4 and the fibroblast activation protein (FAP) which additionally express the pro-inflammatory cytokines IL7 and CCL19, or IL12 to overcome the immunosuppressive environment associated with the tumor (Table 2).[284] Of note, Nectin 4 is a protein which is highly expressed on the surface of various carcinomas and which plays a key role in cancer occurrence and metastasis. Similarly, FAP is highly expressed in tumor stroma.

Combinations of immunotherapeutic strategies

Since cancer immune response involves a coordinate action of diverse immune cells and pathways (Figure 1), it seems highly relevant to combine different strategies acting at various levels of the cancer-immunity cycle to reach superior therapeutic efficacy.[11,285–288] In this line, some clinical investigations are evaluating *i.t.* delivery of various immunomodulatory agents aiming at reshaping the tumor microenvironment in a way that support the promotion/reinstatement of cancer immunosurveillance (Table 2).

Autologous immune cells are infused locally together with immunostimulatory drugs in three recent trials. NCT04952272 will assess in advanced solid cancers the incidence of side effects and primary efficacy of local injection of CpG ODNs, together or not with CAR-T cells releasing anti-OX40. They will be administered consecutively to either microwave ablation or intratumor injection of beads eluting chemotherapeutic compounds that aim to induce the release of tumor-specific antigens. The Phase I trial NCT03707808 will combine *i.t.* injection of autologous $CD1c^+$ myeloid DCs with *i.t.* ipilimumab plus avelumab along with systemic nivolumab for the treatment of solid tumors and metastases to soft tissue. This completed trial demonstrated that *i.t.* injection of autologous $CD1c^+$ myeloid DCs with *i.t.* co-injection of ipilimumab and avelumab is safe and induced early signs of antitumoral activity in pre-treated patients.[289] Similarly, the Phase II clinical trial NCT04571632 will use SBRT along with *i.v.* pembrolizumab, with or without *i.t.* avelumab plus ipilimumab plus $CD1c^+$/$CD141^+$ myeloid DCs[290] in NSCLC.

NCT03739931 is a dose-escalation study of *i.t.* mRNA-2752 in patients with relapsed/refractory solid malignancies or lymphoma. mRNA-2752 consists of mRNAs encapsulated in lipid nanoparticles and which encode the human immune checkpoint OX40L, and the cytokines IL23 and IL36γ. This cocktail will be administered alone and in combination with durvalumab.[291] At all doses studied, the combinatorial treatment was tolerated and associated with tumor shrinkage. Analyses of tumor and plasma biomarkers suggested a sustained immunomodulatory effect that included an elevation of IFNγ, TNFα, and PDL1 levels. These data comfort the application of mRNA-2752 in association with anti-PDL1.[291] Another mixture of three mRNAs, referred to as TriMix, is studied *i.t.* in a Phase I trial prior to surgery or neoadjuvant chemotherapy in early-stage breast cancer patients (NCT03788083). TriMix encodes the immune checkpoints CD70 and CD40L, and a constitutively active TLR4 which promotes DC activation and support T cell priming.[93,292]

Other trials are testing *i.t.* administration of PRR agonists along with other types of immunotherapies. In NCT03928275, response to intralesional delivery of the BCG strain TICE[293] and recombinant IL2 aldesleukin will be measured in participants with cutaneous metastatic melanoma. As another example, in order to expand the efficacy of the TLR4 agonist MPL, a stable emulsion of glucopyranosyl lipid A (GLA-SE) was developed. The Phase I NCT03982121 aimed to determine the MTD, RP2D, and toxicity profile of *i.t.* injection of GLA-SE and ipilimumab in combination with *i.v.* administration of nivolumab and the FOLFOX chemotherapy regimen (i.e. folinic acid, fluorouracil, and oxaliplatin) for the treatment of colorectal liver metastases. Unfortunately, NCT03982121 never began because of withdrawal of the industrial partner. Similarly, in the Phase I trial NCT04270864, ipilimumab and the TLR9 agonist tilsotolimod will be administered *i.t.* in combination with *i.v.* nivolumab in patients with advanced cancers.

The Phase Ib/II trial NCT04387071 studies the side effects and best dose of the VLP-encapsulated CMP-001 administered *i.t.* along with the agonistic anti-OX40 mAb INCAGN01949 in participants with advanced pancreatic cancer and other cancers except melanoma. NCT03831295 evaluates *i.t.* plus *i.v.* BMS-986178 (anti-OX40) along with *i.t.* SD-101 in treating patients with solid malignancies that have metastasized. The Phase I trial NCT03410901 will assess DLT, ORR, and PFS of the same regimen of *i.t.* immunotherapies in combination with radiotherapy in patients with low-grade B-cell non-Hodgkin lymphomas.

Synergistic combinations may also allow to reduce the dose of drugs injected, which both reduce costs and potential adverse events. In this line, CIVO (i.e. Comparative In Vivo Oncology) is an arrayed microinjection technology implanted in a patient's tumor that simultaneously and locally assess tumor responsiveness to microdoses of multiple drugs.[294,295] Three early Phase I clinical trials are currently using this device. First, NCT04272333 studies tumor microenvironment changes following *i.t.* microdose injection of the TLR8 agonist motolimod as a single agent or concomitant with *i.t.* nivolumab in patients with HNSCC. Second, NCT04065555 aims to study the tumor microenvironment modulations induced by TAK-981, a SUMOylation inhibitor shown to activate IFN signaling,[296] as single agent or combined with *i.t.* cetuximab or avelumab in HNSCC. Finally, in the early Phase I NCT04541108, cancer patients will receive intratumoral microdoses of BMS-986299 (NLRP3 agonist), relatlimab (anti-LAG3) or ipilimumab, either as single agents or combined with *i.t.* nivolumab (experimental arm 1). CIVO will also be applied to deliver intralesional microdoses of the STING agonist TAK-676, carboplatin, 5-fluorouracil, or paclitaxel as single agents or in combination (experimental arm 2). In the third experimental arm of NCT04541108, local delivery of microdoses of pembrolizumab will be studied either alone or in association with either MK-0482 or MK-4830, which are antagonists of the inhibitory immune receptors immunoglobulin-like transcript (ILT)-3 and ILT-4, respectively. These treatments will be administered prior to scheduled surgical biopsy or tumor resection surgery. Outcome measures of NCT04541108 will consist in detecting cell death and immune cell biomarkers and determining the relationship between the drugs injected and the incidence of adverse events.

Concluding remarks

Intratumoral immunotherapy has an immediate impact on cancer cells. It locally reshapes the tumor microenvironment in a way that supports cancer immunosurveillance, allowing to reinvigorate tumor-specific immunity. Additionally, this delivery approach offers the advantage to reduce systemic leakage of the therapeutic agent and thus to prevent off-target toxicity. Furthermore, this administration route requires a lower dose to impede tumor progression which opens the gate for drug combinations. Additionally, several therapeutic associations have demonstrated superior efficacy such as ICIs combined to either PRR agonists or ICD-inducing chemotherapy or radiotherapy.[195,297,298] Such regimens seem particularly promising since the anticancer immune response involves the coordinated recruitment and actions of multiple immune effectors, which each incarnates a druggable target for accessing optimized efficacy.

However, intratumoral injection suffers from technical challenges. The targeted tumor must have a sufficient size (>1 cm) to ensure injectability with a needle, especially when the lesions are poorly accessible.[91] It is therefore mainly used for subcutaneous and mucosal lesions or superficial lymph nodes, as highlighted by the numerous clinical trials which are testing *i.t.* immunotherapy in melanoma. Conversely, it is technically more challenging to locally deliver immunotherapies to deeper tumors, as it requires imaging or endoscopic guidance, with associated burden such as repeated x-ray exposure and long procedure. Imaging with ultrasound and/or computed tomography enables to guide needle positioning, but also to monitor tumor size evolution and the effects of the treatment on the tumor microenvironment. For most intraperitoneal lesions and tumors of the central nervous system, imaging is not even sufficient. In this setting, *i.t.* injection requires surgery, which inherently limits the repeatability of injections along the time.[91]

Different questions also remain to be addressed before implementing the technique on a wider scale. Is there a different response if the agent is injected in a metastasis rather than in the primary tumor? Should all metastases be treated to target a high diversity of tumor antigens and elicit broader polyclonal antitumor response? The dose of the agents to administer also remains to be determined; a simple conversion from the systemic dose cannot be applied due to high heterogeneity among the treatments regarding absorption, distribution, and drug interactions. Moreover, the repartition of the drug into the tumors is not uniform and some very dense tumors are difficult to inject. Depending on the pharmacokinetics of the agent, it may leak out in the blood stream and cause unexpected systemic adverse effects. Importantly, the local trauma induced by the needle may have a deleterious impact on the treatment. Finally, local anesthetic administration is required prior to intratumoral injection, and their effects on the tumor microenvironment as well as on the stability and efficacy of the immunotherapeutics is not well known to date.[91] Ongoing and future clinical investigations will have to address these points in order to define the recommendations for intratumoral immunotherapy over systemic treatment, and for optimizing the efficacy of such local monotherapies or combinatorial regimens.

Abbreviations

BCG, Bacillus Calmette–Guérin; CAR-T, chimeric antigen receptor T cell; CIVO, Comparative In Vivo Oncology; CpG, cytidine phosphate guanosine; CSCC, cutaneous squamous cell carcinoma; CTL, cytotoxic T lymphocyte; DAMP, damage-associated molecular pattern; DC, dendritic cell; FDA, food and drug administration; HNSCC, head and neck squamous cell carcinoma; ICD, immunogenic cell death; ICI, immune checkpoint inhibitor; *i.t.*, intratumoral; *i.v.*, intravenous; mAb, monoclonal antibody; NK, natural killer; ODN, oligodeoxynucleotide; OV, oncolytic virus; PexaVec, pexastimogene devacirepvec; PRR, pathogen recognition receptor;

RP2D, recommended Phase 2 dose; SBRT, stereotactic body radiation therapy; T_H1, type 1 T helper cell lineage; TLR, Toll-like receptor; TSA, tumor-specific antigen; T-VEC, talimogene laherparepvec.

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