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REVIEW

Potentiating intratumoral therapy with immune checkpoint inhibitors: shifting the paradigm of multimodality therapeutics

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Immune checkpoint inhibitors (ICIs) have revolutionized oncology, yielding remarkable and durable responses in various cancers. However, a significant proportion of patients develop resistance to ICIs. The tumor microenvironment plays a critical role in immunotherapy resistance, characterized by immune cell composition, regulatory factors, and tumor mutational burden. Intratumoral immunotherapy, involving direct injection of immune-activating agents into tumors, holds promise for converting immunologically 'cold' tumors into responsive 'hot' tumors. This review explores the rationale for combining intratumoral therapies (ITs) with ICIs, highlighting their complementary mechanisms of action and clinical effects. Notable IT approaches include oncolytic viruses, toll-like receptor agonists, and stimulator of interferon gene agonists with ICIs. Overall, combining ITs with ICIs offers a rational strategy to potentiate antitumor immune response and overcome resistance. Further research is needed to optimize the combination strategies, identify biomarkers of response, and establish the safety and efficacy of these novel therapeutic approaches.
Key words: intratumoral therapy, immune checkpoint inhibitors

INTRODUCTION

In recent years, immune checkpoint inhibitors (ICIs) have revolutionized the field of oncology. The utilization of antibodies targeting pivotal immunological checkpoints, such as cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed cell death protein 1 (PD-1) or programmed death-ligand 1 (PD-L1), has led to unparalleled and enduring antitumor responses, yielding substantial survival advantages across a wide range of cancer types.[1] Although the advent of immune checkpoint inhibition has changed the facet of oncology across multiple tumor types, >50% of patients are categorized as non-responders, or they experience disease progression following the initial positive response to ICIs.[2] Immunotherapy resistance is secondary to the features of the tumor microenvironment (TME); the presence of immunosuppressive cells [e.g. regulatory T (Treg) cells], myeloid-derived suppressor cells (MDSCs), or the secretion of immunosuppressive cytokines (e.g. transforming growth factor-β); disruptions in the interferon (IFN) signaling pathway, including alterations in IFN-γ receptor or Janus kinase signaling; and up-regulation of other immune checkpoint receptors, such as T-cell immunoglobulin and mucin domain-containing protein 3 or lymphocyte-activation gene 3.[3] Sustaining a consistently strong and specific immune response in humans utilizing the innate and adaptive branches has continued to be a major hurdle in achieving or maintaining durable responses and an area of unmet need that requires the employment of strategic combinatory molecules and augmented techniques.

Intratumoral therapy (IT) has emerged as a promising approach for modulating the TME and converting immunologically 'cold' tumors into 'hot' tumors. By delivering therapeutic agents directly into the tumor, it can promote immune cell infiltration, enhance antigen presentation, reduce immunosuppressive factors, and induce an inflammatory response, thereby improving the efficacy of ICIs.[4]

Therefore, an imperative exists to devise cogent synergistic regimens that augment T-cell-mediated antitumor efficacy. Multiple studies substantiate the notion that responses to ICIs necessitate the presence of baseline tumor-specific T cells, to be harnessed for immunomodulating agents.[5,6] Utilizing IT provides a means to manipulate the tumor's innate immune system effectively. By activating dendritic cells (DCs) at the tumor site, this approach primes antitumor T cells and ensures the optimal presentation of local tumor antigens/neoantigens, all while mitigating systemic toxicities. Overcoming these obstacles may be achieved through intratumoral administration of agents that

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activate innate immune cells, ultimately promoting the generation or expansion of pre-existing antitumor T cells.[7] The initiation of a systemic immune response can occur through a variety of mechanisms, including the enhanced release and presentation of tumor-specific and associated antigens, immune cell trafficking, and activation and inhibition of immunosuppressive pathways.[8]

While both ITs and ICIs have shown promising results individually, there is a growing interest in combining ITs with ICIs to enhance their respective efficacy. In this literature review, we will discuss the current state of knowledge regarding ITs and ICIs, as well as the potential benefits and challenges of combining these two modalities.

COMBINATION OF INTRATUMORAL THERAPIES AND IMMUNE CHECKPOINT INHIBITORS

Intratumoral application of anti-neoplastic drugs holds promise, as therapeutic concentrations of a drug can be administered locally while mitigating systemic toxicity to modulate in a dose-dependent manner. A systematic literature review was conducted using databases such as PubMed and clinical trial registries, focusing on peer-reviewed publications, preclinical studies, and clinical trial data. Keywords such as 'immune checkpoint inhibitors', 'tumor microenvironment', 'intratumoral immunotherapy', 'resistance mechanisms', and 'HIT' were employed to identify relevant articles. Here, we will briefly describe various ITs that are being explored in combination with ICIs in literature. Tables 1 and 2 summarize the outcomes of various ITs combined with ICIs across multiple tumor types, including melanoma, sarcoma, glioblastoma, breast cancer, and renal cell carcinoma (RCC), mostly in phase I and II trials. A wide range of therapies, such as stimulator of interferon gene (STING) agonists, oncolytic viruses (OVs), toll-like receptor (TLR) agonists, cytokines, bacterial vectors, and nanoparticles, were investigated, with combinations frequently involving pembrolizumab, nivolumab, or ipilimumab. Objective response rates (ORRs) varied, with the highest reported at 83% for IRX-2 [physiologic doses of interleukin 2 (IL-2), IFNγ, and other cytokines derived from activated donor lymphocytes, administered with low-dose cyclophosphamide] in triple-negative breast cancer (TNBC) and the lowest at 0% for IL-2 with ipilimumab in melanoma. Notable responses included talimogene laherparepvec (T-VEC) achieving a 48.6% ORR in advanced melanoma and bempegaldesleukin showing a 52.6% ORR in melanoma with an overall survival (OS) of 30.9 months. Median duration of response (DoR) ranged from 5.8 months [a genetically modified herpes simplex virus type 1 (HSV-1) that expresses human granulocyte-macrophage colony-stimulating factor, fusogenic gibbon ape leukemia virus glycoprotein with the R sequence deleted (GALV-GP-R-), and an anti—CTLA-4 antibody-like molecule known as RP2 in melanoma] to 56.1 weeks (T-VEC in sarcoma), and OS was as high as 12.5 months for glioblastoma treated with DNX-2401 (tasadenoturev; Delta-24-RGD). Most therapies demonstrated manageable safety profiles with minimal dose-limiting toxicities (DLTs). These findings highlight the potential of ITs to improve immunotherapy outcomes across a range of cancers, with promising efficacy and tolerability.[9,10]

Rationale for combination therapy

The rationale for combining ITs with checkpoint inhibitors stems from their complementary mechanisms of action and the desire to enhance the antitumor immune response as depicted in Figure 1. Here are some key aspects of the rationale:

Localized immune activation. ITs, such as OVs, TLR agonists, or immune stimulants, are administered directly into the tumor site. These agents induce localized inflammation, immune cell activation, and release of tumor antigens. This leads to the recruitment and activation of immune cells within the TME.[36]

Overcoming immune suppression. Tumors often employ various immune evasion strategies, including up-regulation of immune checkpoint molecules such as PD-1 and CTLA-4. Checkpoint inhibitors, such as anti-PD-1 or anti-CTLA-4 antibodies, can block these inhibitory signals and reinvigorate exhausted T cells, allowing them to mount a more potent antitumor immune response.[37] In the study by Ma et al., researchers investigated the combined effect of a CD40 agonist and a PD-1 antagonist in mouse models of nonimmunogenic tumors. They observed that this combination therapy led to a significant increase in CD8+ T-cell infiltration within the TME, with a 3.5-fold rise compared with controls. Additionally, there was a notable reduction in granulocytic MDSCs by 50%, indicating a shift toward a more immunogenic tumor milieu. These changes correlated with enhanced antitumor activity, resulting in a 60% decrease in tumor volume relative to untreated groups. The findings suggest that targeting both CD40 and PD-1 pathways can effectively reprogram the TME, facilitating robust T-cell-mediated anticancer responses.[38]

Synergy and complementary effects. ITs can create a more immunogenic TME by enhancing antigen presentation, promoting immune cell infiltration, and modulating the immunosuppressive tumor milieu. This can prime the immune system and create a favorable setting for checkpoint inhibitors to enhance the activation and expansion of tumor-specific T cells.[39] van Hooren et al. evaluated the effects of intratumoral administration of anti-CTLA-4 antibodies, both alone and combined with systemic anti-PD-1 therapy, in a murine bladder cancer model. Monotherapy with intratumoral anti-CTLA-4 led to a significant reduction in tumor growth, with a 50% decrease in tumor volume compared with controls. When combined with systemic anti-PD-1, the treatment achieved complete tumor regression in 60% of the mice. This combination therapy also resulted in a 2.5-fold increase in tumor-infiltrating CD8+ T cells, indicating enhanced antitumor immune responses. These findings suggest that localized checkpoint inhibition, particularly when combined with systemic therapies, can effectively stimulate immune-mediated tumor control.[40]

Table 1. Phase I studies: clinical evidence of synergy between various classes of intratumoral therapies with immune checkpoint inhibitors

Tumor type	No. of patients	Phase	Intratumoral drug	Class of drug	Immunotherapy drug	Prior ICI exposure	ORR	Median DoR	Median overall survival	Median progression-free survival	Trial
Multiple tumor histologies	25	I	MK-1454 i.t. (10-3000 μg) q1w × 9 for 3 cycles and then q3w thereafter	STING agonist	Pembrolizumab at 200 mg i.v. q3w	N/A	24%	N/A	N/A	N/A	NCT03010176[11]
Primary or metastatic pleural malignancy	27	I	Intrapleural delivery of mesothelin-targeted CAR-T cells (3e5/kg to 6e5/kg) + single dose of cyclophosphamide (1500 mg/m^2) i.v.	CAR-T cell	Pembrolizumab at 200 mg i.v. q3w	N/A	12.5% (2 PRs)	N/A	23.9 months	N/A	NCT02414269[12]
Multiple tumor types	16	I	*C. novyi*-NT was administered on day 8 across 4 dose cohorts (3×10^4 to 100×10^4 spores)	Bacterial vector	Pembrolizumab at 200 mg i.v. q3w	Yes	25%	8.18 months	N/A	N/A	NCT03435952[13]
Multiple tumor types	28	I	BO-112 1 mg i.t. q2w	TLR3 agonist	Nivolumab 360 mg i.v. q3w	Yes	10.7% (3 PRs)	N/A	N/A	N/A	NCT02828098[14]
Advanced breast cancer	4	I	CAdVEC i.t.	OV	Pembrolizumab at 200 mg i.v. q3w	N/A	75% (3 PRs)	N/A	N/A	N/A	NCT03740256[15]
Multiple tumor types	106	I	Weekly MIW815 (50-3200 μg) i.t. on a 3-weeks-on/1-week-off schedule or q4w	STING agonist	Spartalizumab 400 mg i.v. q4w	Yes	10.4% (10 PR; 1 CR)	11.5 months	N/A	1.9 months	NCT03172936[16]
Advanced melanoma	91	I	RP1 i.t. 10^6 PFU/ml once f/b q2w at 10^7 PFU/ml for ≤8 total cycles (≤10 ml/dose)	OV	Nivolumab 360 mg i.v. q3w	Yes	37.4%	N/A	N/A	N/A	NCT03767348[17]
Advanced melanoma	17	I	RP2 i.t. 10^6 PFU/ml once followed by up to 7 additional i.t. doses at 10^7 PFU/ml	OV	Nivolumab 360 mg i.v. q3w	Yes	27.2%	5.8 months	N/A	N/A	NCT04336241[18]
Multiple tumor types	9	I	NBTXR3 i.t. + SBRT	Nanoparticle	Nivolumab or pembrolizumab	Yes	40%	N/A	N/A	N/A	NCT03589339[19]
Advanced HNSCC	16	I	NBTXR3 i.t. + SBRT	Nanoparticle	Nivolumab or pembrolizumab	Yes	31.3%	14.8 months	N/A	N/A	NCT03589339[20]
Stage II/III TNBC	12	I	IRX-2 arm: 1 ml SQ × 2 daily for 10 days + cyclophosphamide 300 mg/m^2 i.v. × 1	Cytokine	NACT + pembrolizumab	No	83% pCR	N/A	N/A	N/A	NCT04373031[21]
Advanced leiomyosarcoma	11	I	Trabectedin i.v. (1.2 mg/m^2 q3w) + T-VEC i.t. 1×10^8 PFU/ml q2w	OV	Nivolumab i.v. 3 mg/kg q2w	N/A	18.2% (2 PRs)		18.2 months	7 months	NCT03886311[22]
Advanced melanoma	21	Ib	T-VEC i.t. ≤ 4 ml (10^6-10^8 PFU/ml) on day 22 and then q2w	OV	Pembrolizumab at 200 mg i.v. q3w	No	48%	NR	NR	NR	NCT02263508[23,24]
Recurrent glioblastoma	49	I/II	Single dose of DNX-2401 i.t. by stereotactic injection (5×10^8, 5×10^9, and 5×10^{10} v.p.)	OV	Pembrolizumab at 200 mg i.v. q3w starting 7 days after DNX-2401	No	10.4%	9.4 months	12.5 months	N/A	NCT02798406[25]

Continued

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Table 1. Continued

Tumor type	No. of patients	Phase	Intratumoral drug	Class of drug	Immunotherapy drug	Prior ICI exposure	ORR	Median DoR	Median overall survival	Median progression-free survival	Trial
Follicular lymphoma	52	I/II	G100 i.t. dose escalation (5-20 μg/dose)	TLR4 agonist	Pembrolizumab at 200 mg i.v. q3w	No	33.3%	18.4 months	NR	11.1 months	NCT02501473[26]
Advanced melanoma	21	I/II	PV-10 (10% rose bengal disodium) q3w for 5 cycles	Small-molecule drug	Pembrolizumab at 200 mg i.v. q3w	No	67%	N/A	NR	N/A	NCT02557321[27]

CAR-T cell, chimeric antigen receptor T cell; CR, complete response; DoR, duration of response; f/b, followed by; HNSCC, head and neck squamous-cell carcinoma; ICI, immune checkpoint inhibitor; i.t., intratumoral; i.v., intravenous; N/A, not available; NR, not reached; ORR, objective response rate; OV, oncolytic virus; pCR, pathological complete response; PFU, plaque-forming units; PR, partial response; SBRT, stereotactic radiotherapy; SQ, subcutaneous; STING, stimulator of interferon genes; TLR, toll-like receptor; TNBC, triple-negative breast cancer; T-VEC, talimogene laherparepvec; v.p., ventriculo-peritoneal; w, weekly.

Localized and systemic effects. While ITs primarily target the tumor site, they can also elicit systemic immune responses, including the activation of circulating immune cells or the induction of abscopal effects. Combining checkpoint inhibitors with ITs can enhance both local and systemic immune responses, potentially providing a broader antitumor effect.[10] In the study by Francis et al., researchers investigated the effects of locoregional delivery of ICIs directly to tumor-draining lymph nodes (TDLNs) in preclinical models. They administered anti-PD-1 and anti-CTLA-4 antibodies via peritumoral injection, achieving a 10-fold higher concentration of ICIs in TDLNs compared with systemic administration. This targeted approach led to a significant increase in effector T-cell activation, with a 3.5-fold rise in CD8+ T-cell proliferation within the TDLNs. Consequently, tumor growth was markedly reduced, showing a 60% decrease in tumor volume relative to controls. Importantly, this method minimized systemic exposure, resulting in a five-fold reduction in circulating ICIs, thereby potentially lowering the risk of off-target effects. These findings suggest that delivering ICIs directly to lymph nodes can enhance antitumor immunity while reducing systemic toxicity.[41]

Personalized approaches. The combination therapy allows for a more personalized treatment approach, as different ITs can be tailored to target specific tumor types or genetic alterations, while checkpoint inhibitors can be used more broadly to target immune checkpoint pathways.

Multiple preclinical studies have demonstrated synergy between both therapeutics. Ager et al. examined the combination of CTLA-4, PD-1, and 4-1BB antibodies with myeloid agonists (STING, FLT3) in a prostate cancer xenograft. They administered STING agonist cyclic di-GMP (CDG) or Flt3 ligand (Flt3L) directly into the tumor. Intratumoral CDG or Flt3L enhanced the effectiveness of systemic triple checkpoint modulation, curing 75% of mice with bilateral prostate cancer. Intratumoral CDG also triggered abscopal immunity, regressing tumors at distant sites. The study highlights the synergy between intratumoral myeloid agonists and systemic T-cell checkpoint modulation, offering the potential for controlling cancer spread beyond the treated tumor area.[42]

By combining ITs with checkpoint inhibitors, the goal is to enhance the antitumor immune response, overcome immune evasion mechanisms, and improve patient outcomes. The rationale lies in leveraging the localized effects of ITs to create an immunogenic microenvironment and the systemic effects of checkpoint inhibitors to unleash and sustain the antitumor immune response.

Clinical activity of various intratumoral therapeutics and immune checkpoint inhibitors

Nucleic acid sensors

TLR agonists. Nuclear acid sensors play a vital role in the innate immune system by recognizing both pathogen-associated molecular patterns and damage-associated

Table 2. **Phase II/III studies: clinical evidence of synergy between various classes of intratumoral therapies with immune checkpoint inhibitors**

Tumor type	No. of patients	Phase	Intratumoral drug	Class of drug	Immunotherapy drug	Prior ICI exposure	ORR	Median DoR	Median overall survival	Median progression-free survival	Trial
Advanced sarcoma	12	II	T-VEC i.t. (first dose, $\leq$4 ml $\times$ 10^6 PFU/ml; second and subsequent doses, $\leq$4 ml $\times$ 10^8 PFU/ml	OV	Pembrolizumab at 200 mg i.v. q3w	Yes	35% (7 PRs)	56.1 weeks	NR	17.1 weeks	NCT03069378[28]
Advanced sarcoma	39	II	Trabectedin i.v. (1.2 mg/m^2 q3w) + T-VEC i.t. 1 $\times$ 10^8 PFU/ml q2w	OV	Nivolumab i.v. 3 mg/kg q2w	N/A	7.7%	N/A	19.3 months	7.8 months	NCT03886311[29]
Advanced melanoma	15	II	IL-2 i.t. at 9 MIU twice weekly $\times$ 4 weeks	Cytokine	Ipilimumab (3 mg/kg) four times q3w i.v.	No	0%	N/A	N/A	N/A	NCT01480323[30]
Advanced melanoma	49	II	Tilsotolimod 8 mg i.t. weekly $\times$ 3 weeks f/b q3w $\times$ 3 cycles f/b maintenance with 3 doses q6w (total 9 doses)	TLR agonist	Ipilimumab (3 mg/kg) four times q3 weekly i.v.	Yes	22.4 (2 CRs; 9 PRs)	11.4 months	21 months	5.1 months	NCT03445533[7]
HNSCC	51	II	SD-101 i.t. weekly $\times$ 4 doses and then every 3 weeks $\times$ 7 doses	TLR agonist	Pembrolizumab at 200 mg i.v. q3w	No	23.5%	7 months	NR	2.35 months	NCT02521870[31]
Advanced melanoma	198	II	T-VEC (10^6-10^8 PFU/ml) on week 1, week 4, and q2w i.t. thereafter	OV	Ipilimumab (3 mg/kg) q3w starting day 1, week 6, up to 4 doses	Yes	39%	N/A	NR	13.5 months	NCT01740297[32]
Advanced melanoma	41	II	Bempegaldesleukin 0.006 mg/kg i.t.	Cytokine	Nivolumab 360 mg i.v. q3w for $\leq$2 years	N/A	52.6% (13 CRs)	N/A	NR	30.9 months	NCT03635983[33]
Advanced RCC	49	II	Bempegaldesleukin 0.006 mg/kg i.t.	Cytokine	Nivolumab 360 mg i.v. q3w	No	34.7% (3 CRs)	N/A	NR	7.7 months	NCT03729245[34]
Advanced melanoma	692	III	T-VEC i.t. $\leq$4 $\times$ 10^6 PFU followed by $\leq$4 $\times$ 10^8 PFU 3 weeks later and q2w until dose 5	OV	Pembrolizumab at 200 mg i.v. q3w	No	48.6%		NR	14.3 months	NCT02263508[35]

CR, complete response; DoR, duration of response; f/b, followed by; HNSCC, head and neck squamous-cell carcinoma; ICI, immune checkpoint inhibitor; i.t., intratumoral; i.v., intravenous; N/A, not available; NACT, neoadjuvant chemotherapy; NR, not reached; ORR, objective response rate; OV, oncolytic virus; PFU, plaque-forming units; PR, partial response; RCC, renal cell carcinoma; TLR, toll-like receptor; T-VEC, talimogene laherparepvec; w, weekly.

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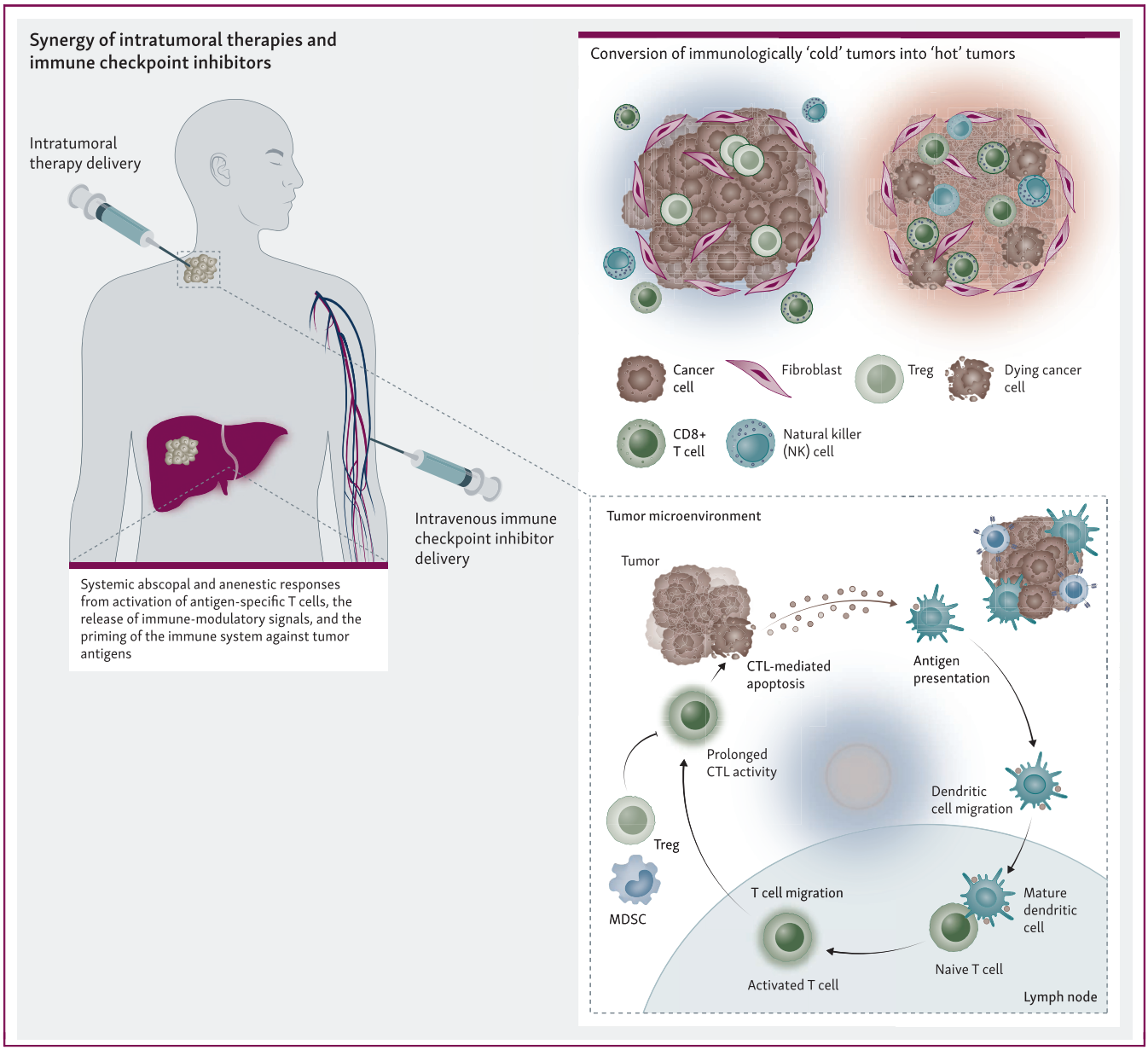

Figure 1. Synergy between intratumoral therapies and immune checkpoint inhibitors. The molecular synergy between intratumoral therapy and immune checkpoint therapy harnesses local immune activation and tumor antigen release, overcoming immunosuppressive mechanisms and checkpoint blockade, and amplifying antitumor immune responses for potent and durable cancer control.
CTL, cytotoxic T lymphocyte; MDSC, myeloid-derived suppressor cell; Treg, regulatory T.

molecular patterns.[43] Upon detection, pattern-recognition receptors (PRRs) initiate signaling pathways that result in the synthesis of proinflammatory cytokines and chemokines, including type-1 IFN. This process leads to the activation of antitumor immune responses, promoting an immune-mediated defense against tumors.[44] The discovery of the immunostimulatory properties of nuclear acid sensor agonists has led to their significant advancement and implementation in clinical settings. Among PRRs, TLRs have been extensively studied. TLR-9, expressed in immune cells, including DCs, macrophages, B cells, and plasmacytoid cells, plays a crucial role in activating innate and adaptive immune responses. TLR-9 signaling induces the production of IFN and proinflammatory cytokines while also stimulating DCs for antigen presentation to cytotoxic T cells.[44,45]

Numerous clinical studies are currently investigating TLR-9 treatments in combination with ICIs. Among them, tilsotolimod (IMO-2125) has made notable progress and has already undergone phase III clinical trials in combination with ipilimumab. While phase II results demonstrated an ORR of 38% in advanced ICI-refractory melanoma, the phase III clinical trial, ILLUMINATE-301, evaluating the therapeutic potential of tilsotolimod in combination with ipilimumab compared with ipilimumab monotherapy, did not demonstrate the achievement of its primary endpoint, namely the ORR, in patients with melanoma refractory to anti-PD-1 treatment (8.8% versus 8.6%).[46]

SD-101 is a synthetic oligonucleotide designed to activate TLR-9 signaling and stimulate the immune system. In a phase Ib basket study, SD-101 combined with pembrolizumab in tumors resistant to ICI demonstrated an ORR of 15% among 13 participants.[47] In ICI-refractory advanced head and neck squamous-cell carcinoma (HNSCC), SD-101, another TLR-9 agonist along with pembrolizumab, demonstrated an ORR of 24% with increased responses in patients who were human papillomavirus (HPV) positive with no correlation with PD-L1 or IFN-γ before therapy.[31]

Preclinical investigations indicate that local administration of CMP-001, a virus-like particle containing a TLR-9 agonist (CpG-A), activates TLR-9 and CD32, facilitating DC internalization. In an early-phase basket trial, intratumoral vidutolimod (CMP-001) with pembrolizumab showed manageable safety, durable responses in 25% of patients, and comparable tumor reductions in treated and untreated lesions.[48] Building on this impetus, the phase Ib study of vidutolimod and pembrolizumab compared with vidutolimod monotherapy showed an ORR of 23.5% versus 20% and a median DoR of 25.2 versus 5.6 months in patients who previously progressed on anti-PD-1 therapy.[49] In a phase II neoadjuvant study in high-risk resectable melanoma, vidutolimod and nivolumab yielded a pathological complete response (pCR) in 47% of resectable melanoma patients, with a major pathological response observed in 57% of patients.[50,51]

Among TLR3 agonists, poly-ICLC (Hiltonol) and BO-112 are two promising agents undergoing clinical investigation in combination with tremelimumab/durvalumab, pembrolizumab, and nivolumab in various early-phase trials. Intratumoral BO-112 and pembrolizumab demonstrated an ORR of 30% in a single-arm phase II trial in advanced ICI-refractory melanoma.[52] Unfortunately, in the TLR7/8 agonist space, MEDI9197 (3M-052), NKTR-262, LHC-165, and CV8102 failed to produce clinical activity in early-phase trials, and further development of these agents is halted.

A phase I/II trial evaluated the safety, tolerability, and early efficacy of the TransCon TLR7/8 agonist as monotherapy and in combination with pembrolizumab in patients with advanced solid tumors. As of 27 May 2022, 19 participants were enrolled: 9 received monotherapy (3 at 0.3 mg, 6 at 0.5 mg), and 10 were treated with the combination therapy (3 at 0.3 mg, 7 at 0.5 mg). A total of 22 treatment-related adverse events (TRAEs) were reported, all of grade 1 or 2, with injection site reactions (59%) and fever (9%) being the most frequent. Pharmacokinetic analysis showed minimal systemic exposure to resiquimod, with a mean half-life of 9 days, and no observed interactions with pembrolizumab.[53] These initial findings suggest that the therapy is well tolerated and merits further exploration in patients with metastatic solid tumors.

STING agonists. The STING pathway is primarily activated by the presence of cytosolic DNA in the cell. When the cell detects the presence of DNA from viral or bacterial sources within the cytoplasm, it triggers the activation of the STING pathway. Upon activation, STING undergoes a conformational change and recruits and activates downstream signaling molecules, such as TANK-binding kinase 1 and interferon regulatory factor 3 (IRF3). This leads to the phosphorylation and nuclear translocation of IRF3, which promotes the transcription of type-1 *IFN* and other immune response genes.[54,55] Desbien et al. conducted preclinical studies showing that intratumoral stimulation of STING using a synthetic cyclic dinucleotide resulted in the activation of antitumor CD8 T-cell immunity. This approach synergistically enhanced the effectiveness of checkpoint inhibitors by promoting CD8+ T-cell-mediated control of non-injected tumors. The observed improved tumor control was correlated with augmented functional characteristics of cytotoxic T cells locally. Additionally, combining MIW815 (ADU-S100) with ICI demonstrated enhanced long-lasting immune responses in a tumor model with low immunogenicity.[56] MIW815 (ADU-S100) and MK-1454 (Ulevostinag) are the most well known in this class undergoing clinical testing with ICIs. The combination of MIW815 (ADU-S100) and spartalizumab showed manageable safety but with modest antitumor efficacy in an early-phase basket trial, including melanoma where partial response (PR) was seen in two patients and TNBC where two patients had PR and one had complete response (CR).[57] Interim findings from a dose-escalation trial evaluating the efficacy of MK-1454 (Ulevostinag) in patients with advanced solid tumors or lymphoma demonstrated within the combination therapy group with pembrolizumab that 24% of patients ($n = 6/25$) exhibited PRs, with >80% response locally and systemically. Disease control was achieved in 48% of patients in the combination arm. Finalized results are pending as trial accrual has been completed.[11]

RIG-I-like receptors. Retinoic acid-inducible protein I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5) are cytoplasmic RNA helicases belonging to the RIG-I-like receptor family mediating innate immunity to viral infections.[58] They detect viral double-stranded RNA and signal through the mitochondrial antiviral signaling protein leading to the production of type-1 IFN.[59] RIG-I activation may lead to the activation or modulation of STAT-1 signaling, potentially influencing downstream immune and inflammatory responses via proinflammatory cytokine production.[58] The synthetic oligoribonucleotide RGT100 (MK-4621) stimulates IFN production. Translationally, intratumoral RGT100/MK-4621 demonstrated significant antitumor efficacy, impacting both the treated and untreated contralateral tumor sites. This therapeutic approach induces a potent and persistent IFN-α and IFN-β response. Notably, the reduction of natural killer (NK) cells hampers the antitumor activity elicited by RGT100 (MK-4621). RGT100 (MK-4621) was examined in a phase I/II study along with pembrolizumab where three PRs (10%) were observed. Translationally, it was noted to up-regulate gene expression of *IFN* cascade, but further development has been terminated.[60]

Cytokines. Cytokines have been employed in the field of cancer therapeutics extensively and have claimed a pivotal space in the field of immuno-oncology.

The role of IL-2 cytokine in cancer has been extensively investigated. Human IL-2, with a molecular weight of 15.5 kDa and composed of 133 amino acids, possesses a Stokes radius of ~3 nm.[61,62] Notably, IL-2 exhibits intriguing immunomodulatory properties, capable of both stimulating cytotoxic effector cells and suppressing Treg cells.[63] Despite being available for several decades, the systemic use of IL-2 is limited secondary to significant adverse effects like capillary leak syndrome, influenza-like symptoms, fever, hypotension, organ dysfunction, and neurological effects. Nevertheless, intratumoral IL-2 has emerged as a promising approach to mitigate these adverse effects by exploiting the intratumoral therapeutic index while mitigating systemic toxicities. Local administration of IL-2 produced local responses predominantly.[63] To tackle the limitations of intratumoral IL-2 treatment in demonstrating distant responses, a phase I study was conducted to investigate the combination of intratumoral ipilimumab and IL-2 in patients with unresectable stage III/IV melanoma. Antitumor efficacy in injected lesions was seen in 67% (8/12), while systemic responses were observed in 88.9% (8/9) of participants. Translational work also provided evidence of systemic immune activation with a higher population of cytotoxic T cells, IFN, and granzyme-B.[64]

Nevertheless, cytokines play a crucial role in the regulation of numerous biological pathways, and their intravenous administration can induce significant off-target adverse effects.[65] To confine the expression of cytokines to specific tissues, one strategy involves the incorporation of cytokine-encoding genes into locally administered DNA plasmids, messenger RNA (mRNA) molecules, or viruses. This localized delivery allows for the recombination of cytokines within the targeted site.

SAR441000 is an mRNA-targeted intratumoral therapeutic with IL-12, IFN-α2b, granulocyte—macrophage colony-stimulating factor (GM-CSF), and IL-15 sushi. In a phase I basket trial (NCT03871348), SAR441000 was tested as monotherapy and combined with cemiplimab. During dose escalation, two confirmed responses (one CR; one PR) were noted, while in dose expansion, two confirmed responses (two PRs) were seen in melanoma. Although meaningful clinical responses were observed in 13 patients (32%) involving either injected or non-injected lesions, the responses were primarily localized to the region of treatment, and no significant responses were seen in distant non-injected lesions.[66]

The phase I dose-escalation trial (NCT02555397) assessed the safety and immunological effects of an oncolytic adenovirus (Ad5-yCD/mutTKSR39rep-hIL-12) encoding IL-12, administered intratumorally to 15 patients with localized recurrent prostate cancer (T1c-T2). Doses ranged from 1×10^{10} to 1×10^{12} viral particles, followed by a 7-day course of 5-fluorocytosine and valganciclovir. The therapy was well tolerated, with no DLTs or maximum tolerated dose identified. Most adverse events (AEs) (92%) were grade 1/2 and required no intervention. Adenoviral DNA was detected in only two participants. Serum cytokine analysis showed dose-dependent increases in IL-12, IFN-γ, and CXCL10 in 57%, 93%, and 79% of patients, respectively, with the highest responses in the maximum dose cohort. Immune system activation was evident in peripheral blood mononuclear cells of the highest-dose group, while median prostate-specific antigen doubling time pre- and post-treatment was 1.55 years and 1.18 years, respectively. The findings confirm the safety and immunostimulatory potential of Ad5-yCD/mutTKSR39rep-hIL-12 in localized prostate cancer.[67]

CLN-617, a novel IT delivers IL-2 and IL-12 in a single fusion protein designed for safety and efficacy. This therapeutic includes IL-2, IL-12, leukocyte-associated immunoglobulin-like receptor 2 (to bind collagen and enhance tumor retention), and human serum albumin (to increase molecular weight). In preclinical models, intratumoral administration of a murine surrogate (mCLN-617) eradicated both treated and distant tumors, improved responses to anti-PD-1 therapy, and triggered abscopal effects through cellular immunity and antigen cross-presentation. CLN-617 is now under clinical evaluation for advanced solid tumors in trial NCT06035744.[68]

Another phase I trial (NCT03946800) evaluated MEDI1191, an intratumoral lipid nanoparticle-formulated mRNA encoding IL-12, in combination with durvalumab (anti-PD-L1) in patients with advanced/metastatic solid tumors, including those with subcutaneous/cutaneous and deep-seated lesions. Among 61 patients treated [40 with prior anti-PD-(L)1], no DLTs or maximum tolerated dose was observed. Grade ≥3 MEDI1191-related AEs occurred in 4.9% of patients, including asthenia, pyrexia, and decreased lymphocyte count. Confirmed PRs were observed in five patients across melanoma, sarcoma, breast, and neuroendocrine cancers, with a median DoR ranging from 1.9 to 22.3 months (median not reached). Additionally, serum IL-12 levels increased twofold or more in 91% of patients, accompanied by elevated IFN-γ in 80% and increased CD8+ T-cell infiltration and PD-L1 expression in 41%. These findings demonstrate that MEDI1191 combined with durvalumab is safe, induces systemic and intratumoral immune activation, and shows preliminary antitumor activity.[69]

The phase III PIVOTAL trial (NCT02938299) assessed the efficacy and safety of daromun, an intralesional neoadjuvant therapy combining L19IL2 and L19TNF, in patients with stage III locally advanced melanoma eligible for surgical resection. Conducted at 22 sites in Europe, the study randomized 127 patients to receive up to 4 weekly injections of daromun before surgery and 129 patients to undergo surgery alone. Daromun significantly improved relapse-free survival (RFS), with a median RFS of 16.7 months compared with 6.9 months in the control arm [hazard ratio (HR) 0.59, 95% confidence interval 0.41-0.86, $P = 0.005$, as per central review]. Distant metastasis-free survival also showed notable improvement (HR 0.60, $P = 0.029$), and 21% of patients in the daromun group achieved a complete pathological response. The treatment

was well tolerated, with low-grade local side-effects (14% grade 3) and minimal systemic AEs. These results highlight daromun as a promising neoadjuvant therapy for improving outcomes in patients with resectable melanoma.[70]

Viral vectors. OVs have been under investigation for their potential as anticancer agents for more than a century. While initially studied for their direct tumor-killing abilities, recent research has focused on their capacity to stimulate antitumor immune responses. T-VEC, is an OV-based immunotherapy used for the treatment of advanced melanoma. It is a modified HSV-1 that has been engineered to selectively replicate within tumor cells and produce GM-CSF. T-VEC received approval from the United States Food and Drug Administration (FDA) in October 2015. It was approved for the treatment of unresectable stage IIIB, IIIC, and IV melanoma, making it the first oncolytic viral therapy to be granted FDA approval. T-VEC has exhibited the capacity to provoke regression in injected tumors and trigger an immune-mediated response in non-injected lesions. However, it has limited efficacy in controlling distant disease progression.[71]

Combining T-VEC with ICIs is one strategy to enhance its immunological effects. T-VEC incorporates GM-CSF to activate antigen-presenting cells (APCs) and induce a systemic antitumor response. The presence of GM-CSF enhances regression in non-injected tumors, indicating the importance of both viral oncolysis and GM-CSF-mediated APC stimulation in T-VEC's efficacy.[71] Multiple preclinical studies have shown synergy with intratumoral GM-CSF and CTLA-4 and PD-1 blockade with local and systemic responses holding therapeutic promise for this combination.[72,73] The initial phase Ib trial focused on the combination of T-VEC and ipilimumab in previously untreated patients with stage IIIB-IV melanoma. T-VEC and ipilimumab exhibited an ORR of 50% in 44% of patients with durable responses lasting >6 months.[74] In the subsequent phase II study with 5-year follow-up, T-VEC and ipilimumab showed an ORR of 36% versus 16% in the ipilimumab monotherapy arm among 198 patients.[75]

Additionally, T-VEC has been evaluated in combination with pembrolizumab. In a phase Ib trial in unresectable melanoma, the confirmed ORR was 62% with 50% of participants experiencing CR among 21 patients.[76] However, the phase III trial (MASTERKEY-265: NCT02263508) with T-VEC and pembrolizumab versus placebo plus pembrolizumab in patients with advanced melanoma, involving 692 randomized patients, reported an ORR of 48.6% versus 41.3% with no statistically significant differences observed in progression-free survival (PFS), DoR, and OS.[35] T-VEC and pembrolizumab were explored in a phase II study in undifferentiated pleomorphic sarcoma and myxofibrosarcoma, cutaneous angiosarcoma, epithelioid sarcoma, and undifferentiated sarcoma where the overall best ORR for angiosarcoma was 71% (5/7 patients) with a median PFS of 54 weeks.[77]

Vusolimogene oderparepvec (RP1) is a novel agent utilizing a modified HSV-1 that expresses human GM-CSF and a fusogenic protein (GALV-GP-R$-$). In the phase I/II IGNYTE study in ICI-refractory advanced melanoma (NCT03767348), with 91 patients, the overall ORR was 37.4% with the bulk of responses in the PD-L1-negative tumors. Notably, 18.7% of patients achieved CR. Interestingly, the therapy showed responses in non-injected lesions, even in patients with extensive visceral disease.[17] NCT04336241 is a phase I study of RP2 in combination with nivolumab in a cohort of patients with uveal melanoma. RP2 and RP3 are modified versions of RP1 designed to express supplementary immune-activating proteins. RP2 incorporates a molecule resembling an anti-CTLA-4 antibody, while RP3 expresses the immune co-stimulatory proteins CD40L and 4-1BBL. Notably, RP3 does not include the expression of GM-CSF. Among 14 patients who were ICI refractory, the overall ORR for 14 patients was 28.6% (4/14) with three PRs in the combination arm with a median DoR of 5.8 months.[18] NCT05743270 will explore RP3 in inoperable, previously untreated, high-risk HNSCC eligible for curative intent chemoradiation and will be randomized 1 : 1 to receive RP3 $+$ standard-of-care cisplatin-based chemoradiation, followed by nivolumab compared with chemoradiation.[78]

Intratumoral administration of OrienX010, an oncolytic virotherapy derived from HSV-1 and expressing GM-CSF, was investigated in stage IV melanoma patients with liver metastases receiving toripalimab. Interim results from 29 patients showed an ORR of 20.7% (6/29). The response rate was 31.0% (9/29) for injected lesions, 30.0% (6/20) for non-injected lesions in the liver, and 27.8% (5/18) for extrahepatic metastases. The median PFS was 7.0 months, and the median OS was not reached.[79,80]

T3011, a recombinant HSV-1 OV expressing IL-12 and anti-PD-1 antibody, induces local IL-12 production, enhances immune response, inhibits tumor growth, and promotes anti-angiogenesis. In a dose-escalation study, 6 out of 29 patients were rechallenged by combining T3011 with pembrolizumab, where 1 patient with a durable PR lasting over 8 months correlated with increased CD8$+$ T-cell infiltration.[81]

Pexastimogene devacirepvec (Pexa-Vec; JX-594) is an OV belonging to the class of vaccinia viruses genetically modified with incorporation of the GM-CSF gene and deletion of the thymidine kinase gene that exhibits selective tropism for cancer cells, leading to intracellular replication, cell lysis, and immune system activation.[82] Promising preclinical and clinical data highlighted its safety in hepatocellular carcinoma and colorectal cancer although modest clinical activity only was noted. In the phase I/II study of Pexa-Vec in combination with durvalumab in refractory metastatic colorectal cancer, of 14 patients assessable for response, 1 patient (7%) had confirmed PR.[83] However, the phase Ib study of Pexa-Vec in combination with cemiplimab (REN026; NCT03294083) showed promise in advanced RCC with an ORR of 37.5% (6/16) while grade 3 TRAEs included temperature elevation, influenza-like symptoms, and lung infection.[84]

Bacterial vectors. Following the initial approval of BCG for bladder cancer treatment, there is a growing interest in

exploring the therapeutic potential of other attenuated bacterial strains in the field of cancer therapy. These bacteria utilize a multifaceted approach to engage various PRRs, such as TLR2 and TLR4, and also provide xenoantigens, thereby attracting and activating immune surveillance cells. Consequently, they can induce an antitumor immune response with reduced toxicity.[85] Notably, anaerobic strains, which possess a preference for replicating within tumor environments, have become a focus of several clinical trials.

The intratumoral administration of *Clostridium novyi*-NT, a non-toxic strain of *C. novyi*, in combination with intravenous pembrolizumab, is currently being investigated in patients with advanced solid malignancies. This approach exploits the ability of *C. novyi*-NT to selectively replicate within hypoxic tumor regions, leading to tumoricidal death and inflammation. In the phase Ib basket study, a confirmed overall ORR of 25% was observed, with three PRs and one CR and favorable tolerance. The median DoR among responders was 8.18 months, and 25% had previously received ICIs. The injected tumor size ranged from 1 cm to 11 cm, with a confirmed ORR of 19% in injected lesions.[13]

SYNB1891, a genetically modified variant of the probiotic *Escherichia coli* Nissle 1917, has been developed to generate cyclic dinucleotides in oxygen-deprived conditions. This leads to the activation of STING triggering innate immune activation. The initial clinical trial (NCT04167137) involved patients with advanced, treatment-resistant cancers who received repeated injections of SYNB1891 directly into the tumor site, either as a stand-alone treatment or in combination with atezolizumab. Administering SYNB1891 resulted in the activation of the STING pathway and demonstrated its effectiveness through the up-regulation of IFN, chemokines/cytokines, and T-cell pathway, with four participants who had previously not responded to PD-(L)1 antibodies showing stable disease (SD).[86]

Adoptive cell therapy. Adoptive cell therapy (ACT) is a rapidly evolving field of cancer immunotherapy where donor cells are reconfigured in an *ex vivo* environment, and then systemically infused to the patient. ACT is broadly divided to comprise the well-known class of chimeric antigen receptor T cells (CAR-T cells), tumor-infiltrating lymphocytes (TILs) that have made headway in melanoma, and genetically engineered T-cell receptors.[87] Systemic injection often results in low cell count at lesions, compromising efficacy. In contrast, intratumoral injection delivers effector cells directly to the target site, increasing their concentration and overcoming immunosuppression, thereby enhancing therapeutic outcomes. While CAR-T-cell therapy has demonstrated efficacy in hematologic malignancies, its application in solid tumors has been hindered by challenges such as variable antigen expression, insufficient T-cell infiltration into tumor sites, and suppression within the immunosuppressive TME. To overcome these barriers, a novel approach utilizing regionally administered mesothelin-targeted CAR-T-cell therapy has been developed. In a phase I trial, regionally delivered mesothelin-targeted CAR-T-cell therapy was safe and well tolerated in patients with malignant pleural diseases, with CAR-T cells persisting in 39% of patients for over 100 days. Adding pembrolizumab enhanced outcomes, achieving a median OS of 23.9 months, a 1-year survival rate of 83%, and sustained disease stabilization in several patients, including two complete metabolic responses.[12] These findings suggest significant potential for combining regional CAR-T-cell therapy with PD-1 blockade in solid tumors. Other trials exploring locoregional infusion of ACTs include carcinoembryonic antigen-specific CAR-T cells for adenocarcinoma peritoneal metastases or MUC16-CAR-T cells for ovarian cancer.[88]

Antibodies. Although no promising agents in the space of naked antibodies in combination with ICI have made headway yet, in the conjugated antibodies therapeutics, LMB-100 holds promise.

LMB-100 is an advanced recombinant immunotoxin that combines a humanized anti-mesothelin fragment antigen binding with a modified fragment of Pseudomonas exotoxin A to minimize immune recognition.[89] Its efficacy has been demonstrated in preclinical models expressing mesothelin, including patient-derived xenograft models.[90] A phase I clinical trial confirmed the safety of LMB-100 in various solid tumors (NCT02798536). Jiang et al. noted that treatment with LMB-100 followed by pembrolizumab led to increased tumor responses in participants. The ORR was 40%, with a median OS of 11.9 months. Notably, participants with positive tumor PD-L1 expression exhibited better responses and longer PFS (11.3 months versus 2.1 months) compared with PD-L1-negative participants.[91]

Sotigalimab (APX005M) is a potent CD40-targeting monoclonal antibody (mAb) that activates APCs, promoting cancer-specific T-cell responses. In a randomized phase II trial (NCT03214250) assessing the efficacy of sotigalimab $\pm$ nivolumab combined with gemcitabine and nab-paclitaxel chemotherapy in first-line metastatic pancreatic adenocarcinoma patients, the primary endpoint of 1-year OS was achieved with nivolumab and chemotherapy (57.7% versus historical OS of 35%). However, sotigalimab and chemotherapy (48.1%) and sotigalimab and nivolumab and chemotherapy (41.3%) did not meet the primary endpoint, albeit showing trends toward improved survival.[92]

Cancer vaccines. Therapeutic cancer vaccines are engineered to stimulate adaptive immunity against cancer cells by leveraging tumor-specific and associated antigens. These vaccines rely on the availability of a plentiful source of antigens from the tumor, which are captured and presented to T cells by DCs to initiate an immune response. The effective uptake and presentation of these antigens by DCs are crucial for the success of therapeutic cancer vaccines.[93] Cancer vaccines adopt diverse forms, including isolated antigens, genetically engineered DNA, RNA, or viruses encoding antigenic material, cellular extracts, and DCs. In the pursuit of vaccine optimization, notable efforts are being invested in developing bioinformatics algorithms

capable of forecasting the antigen epitope—major histocompatibility complex molecule binding prediction along with innovative proteogenomic strategies.[93]

VB10.16 is an experimental off-the-shelf therapeutic vaccine that targets precancers and cancers caused by human papillomavirus type 16 (HPV16).[94] VB10.16, in combination with atezolizumab, demonstrated improved patient outcomes in PD-L1-positive, HPV16-positive advanced cervical cancer with a median OS >25 months, in the phase II VB-C-02 trial (NCT04405349). Overall, the ORR was 19% with a 6% CR rate while in the PD-L1-positive population, the ORR was 29% with a CR of 8%. Grade 3 TRAEs related to atezolizumab were observed in 10% of patients.[95]

GX-188E (tirvalimogene teraplasmid) is a DNA vaccine targeting HPV and transcribing E6 and E7 proteins of HPV16/18 strains.[96] Phase II interim analysis results (KEYNOTE-567) where GX-188E was combined with pembrolizumab in patients with HPV16 and/or HPV18-positive advanced cervical cancer demonstrated the best overall response in 5 (10.4%) patients who had a CR and 10 (20.8%) patients who had a PR among 48 assessable patients. The benefit was predominantly seen in patients with PD-L1-positive, HPV16, and squamous-cell carcinoma histology.[97]

The therapeutic DNA vaccine MEDI0457, containing plasmids encoding HPV16/18 E6 and E7 oncogenes along with IL-12 adjuvant, demonstrates safety and induces an immune response against the targeted antigens. In a phase II trial combining MEDI0457 with durvalumab for recurrent/metastatic HPV-associated cancers, the ORR was 21%. Notably, CRs and PRs were observed in cervical, anal, and penile squamous-cell carcinomas. Responders exhibited a median DoR of 16 months. Median PFS was 3.7 months, and OS was 13.5 months. Grade ≥3 AEs occurred in 14% of patients, with manageable toxicities and no study discontinuation required.[98]

Tavokinogene telseplasmid (tavo) is a DNA plasmid that contains genes encoding both p35 and p40 subunits of the human IL-12 protein. These genes are arranged with an internal ribosome entry site and controlled by a single cytomegalovirus promoter. When administered through intratumoral injection and electroporation, the plasmid enters human cells, leading to the production and localized release of functional IL-12 p70 protein leading to potential immunomodulatory and anticancer effects.[99] A phase II trial of tavo in combination with pembrolizumab in immunologically quiescent melanoma demonstrated superior efficacy with an ORR of 41% with 87% of patients experiencing CR. Pharmacodynamic evidence from the study demonstrated T-cell priming, antigen presentation, increased TILs, and expansion of T-cell repertoire leading to cellular immunity activation.[100]

Nanoparticles. NBTXR3 is a radio-enhancing intratumoral therapeutic comprising functionalized hafnium oxide nanoparticles and stimulated by radiotherapy. Study 1100, a phase I trial (NCT03589339), investigates NBTXR3 activated by stereotactic body radiotherapy followed by anti-PD-1 therapy (nivolumab or pembrolizumab) in advanced solid tumors. Escalation cohorts based on injection site [head and neck (H&N), lung, or liver] treated 28 patients. Two DLTs occurred in one patient at the first dose level in the H&N cohort. Grade ≥3 NBTXR3-related AEs occurred in four (14.3%) patients. Among 20 assessable patients, the ORR was 40% with durable CRs, also seen in ICI-refractory population.[19,101] In locoregionally recurrent or metastatic HNSCC, among 16 patients ORR was observed in 31.3% (5/16) of patients with a mean DoR for these 5 patients of 14.8 months.[20] Currently, NBTXR3, radiation, and ICI for the treatment of lung and/or liver or soft tissue metastases from advanced tumors are being explored in a phase I/II study (NCT05039632).

FUTURE DIRECTIONS

IT has emerged as an effective method for locoregional therapy, expanding the repertoire of treatment options. This approach involves delivering immunomodulatory agents directly into the tumor, resulting in higher drug concentrations within the tumor and minimizing unnecessary systemic exposure. Furthermore, intratumoral administration grants immediate entry into the tumor's tertiary lymphoid organs, thereby amplifying the therapeutic efficacy of this strategy.[102] In the context of their mechanism of action, these therapies engage various stages of the cancer immunity cycle. They facilitate antigen presentation and promote the activation of T cells, leading to a comprehensive antitumor immune response that extends beyond the directly treated sites and can be observed systemically. The favorable tolerability profile and the absence of off-target toxicities have made these therapies suitable for clinical use. Their combination with ICIs has shown synergistic effects in clinical settings. However, the practical implementation of these therapies in the clinic requires careful consideration of several factors as depicted in Table 3. These include patient selection criteria, strategies for assessing treatment response, and approaches to optimize local delivery methods, which are essential for enhancing treatment efficacy and ensuring patient safety.

Optimizing treatment delivery and dosing

Preventing the therapeutic diffusion of local mAbs from the tumor tissue is a significant area of inquiry regarding their therapeutic activity. Various pharmaceutical formulations offer potential solutions by enhancing the local drug absorption of intratumoral therapeutics. One approach involves formulating emulsions with ethiodized oil and poly(lactic-co-glycolic acid) nanoparticles for intratumoral injection, enabling a gradual and controlled release of anti-CTLA-4 mAbs. Another strategy entails conjugating mAbs to a highly binding peptide derived from placental growth factor 2, an extracellular matrix component. This conjugation promotes better retention of the mAbs within the tissue and reduces their concentration in the bloodstream. Consequently, this approach has demonstrated promising outcomes in preclinical models, including a reduced incidence of systemic immune-related AEs and enhanced

Table 3. Advantages, disadvantages, and considerations in intratumoral therapy and immune checkpoint inhibitors

Advantages	Disadvantages	Considerations
Universal therapeutic strategy rather than a personalized cancer vaccine approach: not dependent on tumor-specific antigens	Barriers in enrollment due to invasive techniques and frequency of administration	Standardization, synchronization, and timing of biopsies to assess pharmacodynamic response in injected and non-injected lesions
Direct delivery of immunostimulatory drugs to the tumor site enhances therapeutic efficacy	Limited accessibility of injected lesions	Standardizing IT responses and reporting with response criteria for intratumoral immunotherapy in solid tumors in injected and non-injected lesions
Addresses tumor heterogeneity by creating immune reactivity against diverse cancer cell epitopes	Eliciting heterogeneous tumor response locally and systemically	Accessibility and optimization of local drug delivery to each lesion
Versatile partner for synergistic combinatory regimens and multimodality therapeutics	May not be effective as monotherapy to mediate systemic effects	Defining which pharmacokinetic/pharmacodynamic markers could help to determine the appropriate dose and regimen
Allows for sequential pharmacodynamic monitoring for response and resistance	Safety considerations in repeated injections; prior irradiation; site of lesions; vasculature involvement	Translating traditional concepts of trial designs, MTDs, and DLTs to IT
Facilitates optimal dose exposure by increasing bioavailability in the TME while limiting the systemic exposure	Injection site reactions secondary to IT	Determine the OBD rather than the MTD; detection of anti-drug antibodies
Enables testing of potent immune stimulators that may otherwise have prohibitive systemic toxicity	Operator dependence on administration is subject to variability	Guidance for injectable lesion ranking, technical aspects of injection delivery

DLT, dose-limiting toxicity; IT, intratumoral therapy; MTD, maximum tolerated dose; OBD, optimal biological dose; TME, tumor microenvironment.

antitumor efficacy compared with unconjugated mAbs.[10,103] Additional challenges to address include variations in dosing within the same clinical trial, exemplified by MIW815 (ADU-S100). Researchers propose that treatment response may be influenced by drug concentration and the number of injected lesions, underscoring the importance of dose determination based on tumor dimension and administration of the maximum allowable concentration of therapy.[57]

Novel approaches

Delivery challenges caused by intratumoral pressure and immunosuppression from MDSCs in liver tumors have hindered immunotherapy efficacy. The PERIO-01 trial explores pressure-enabled drug delivery of SD-101 via hepatic arterial infusion in combination with ICIs for uveal melanoma liver metastases. The study includes dose-escalation cohorts of SD-101 alone or with nivolumab and ipilimumab. Translationally, serum IL-18 and IFN-γ levels were elevated, while NK cell expansion and drop in monocytic MDSC levels were observed. Genomic profiling revealed decreased ARG-1 and IDO-1 gene levels for up to 3 months. Lower doses of SD-101 with nivolumab demonstrated disease control in five of six patients, with SD lasting a median of 12 weeks.[104]

INT230-6 is a novel formulation of cisplatin, vinblastine, and a tissue dispersion enhancer (SHAO) designed for intratumoral delivery leading to direct cytotoxic effects, immune activation, and apoptosis. IT-01 is an open-label phase I/II study in adults with locally advanced, unresectable, or metastatic solid tumors, including sarcoma studying in combination with ipilimumab. In 29 sarcoma patients, it was reasonably tolerated while INT230-6 along with ipilimumab was associated with immune T-cell infiltration and improved median OS that was not reached even after 1 year when compared with historical control (205 days), particularly when $\geq$40% of the tumor burden was injected.[105]

The phase II NeoIRX trial examined immunologic induction with peri-lymphatic cytokines to enhance pembrolizumab response in stage II/III TNBC. The IRX-2 regimen comprised physiologic doses of IL-2, IFN-γ, and other cytokines derived from activated donor lymphocytes and given with low-dose cyclophosphamide before neoadjuvant chemotherapy and pembrolizumab. The IRX-2 arm achieved an 83% pCR (5/6) compared with a 33% pCR with pembrolizumab alone (2/6). The trial terminated after 12 enrollments secondary to a lack of funding for IRX-2. Encouraging outcomes, supporting further study of peri-lymphatic induction cytokine therapy in stage II/III TNBC, hold promise.[21]

Optimizing treatment sequence

The optimal timing for combining locoregional therapy and immunotherapy, whether given concurrently or sequentially, remains uncertain after local treatment. Although several clinical trials are currently under way to explore the combination of locoregional therapy with immunotherapy, only a limited number of them have incorporated specific considerations regarding the sequence or timing of these therapies.

Nguyen et al. examined the literature to assess the optimal timing of PD-1 blockade in combination with OV therapy and found that the hypothesized most promising treatment strategy thus far involves a sequential approach known as OV run-in followed by combination therapy. In the OV run-in followed by a combination treatment strategy, the initial administration of OV treatment triggers the activation and infiltration of T cells into tumors, resulting in an immunologically active response. However, this immune response can be dampened by the interaction of PD-L1 and the PD-1 receptor on the surface of activated T cells, B cells, NK cells, DCs, and macrophages leading to functional

impairment of T cells. Consequently, after a run-in of OV, it is crucial to simultaneously administer both OV and an ICI. This treatment strategy ensures that the OV sustains T-cell activation and maintains an immune receptive, while ICI inhibition disables checkpoint-mediated T-cell exhaustion.[106]

Since the immunomodulatory effects induced by locoregional therapy have a limited duration, additional research is needed to determine the optimal timing and sequence of combined immunotherapy to enhance treatment effectiveness.

Delineating treatment response

Phase II clinical trials have demonstrated promising results for several intratumoral agents with ICI. However, recent phase III studies, including those involving T-VEC/pembrolizumab and tilsotolimod/ipilimumab, have yielded negative outcomes.[7,35] These results underscore the significance of careful participant stratification and in-depth analysis to discover predictive and prognostic biomarkers associated with outcomes. In evaluating treatment response, various response criteria have been employed in recent clinical trials. Nevertheless, widely used criteria such as Response Evaluation Criteria in Solid Tumours (RECIST) have limitations when applied to intratumoral treatments.[107,108] RECIST v1.1 does not allow for separate assessment of injected and non-injected lesions, while the treatment effect may lead to changes in the size of injected lesions. Immune RECIST attempts to distinguish true progression from pseudoprogression, but it does not comprehensively assess overall tumor response due to potential changes in lesions during progression. Furthermore, radiologic imaging may not capture certain cutaneous lesions, necessitating additional clinical evaluation. In the context of intratumoral immunotherapy, the American Society of Clinical Oncology (ASCO) guidelines recommend the use of response criteria for intratumoral immunotherapy in solid tumors criteria for easy adoption in clinical practice.[109,110]

Combining drugs in synergistic combinations offers the potential to reduce the required dosage, leading to cost reduction and minimizing the occurrence of AEs. To explore this approach, Comparative *In Vivo* Oncology (CIVO) is an advanced platform that enables the concurrent assessment of multiple anticancer agents within a single tumor. This technique involves the direct administration of distinct therapies into different regions of the tumor, facilitating the evaluation and comparison of their efficacy. By analyzing the response of individual tumor regions to various treatments, CIVO offers valuable scientific insights that can inform the development of personalized and targeted therapeutic approaches for cancer.[111] Several phase I clinical trials are currently investigating the effects of intratumoral microdose injections of various drug combinations including TLR8 agonist motolimod, TAK-981, a SUMOylation inhibitor, and MK-0482 or MK-4830, which block the inhibitory immune receptors immunoglobulin-like transcript 3/4.

CONCLUSION

ITs and ICIs have emerged as powerful tools in the fight against cancer. Their synergistic potential holds great promise for enhancing treatment outcomes and overcoming challenges associated with systemic therapies. By directly targeting tumors and activating the immune system, these approaches offer the opportunity for precise and effective cancer treatment. As ongoing research and clinical trials continue to explore the optimal combinations, dosing strategies, and patient selection criteria, the impact of ITs and ICIs is expected to revolutionize a multimodality approach in oncology.

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