ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

IOTECH IMMUNO-ONCOLOGY AND TECHNOLOGY

REVIEW

Regional and intratumoral adoptive T-cell therapy

I. Olivera[1,2]*, I. Etxeberria[3,4], C. Luri-Rey[1,2], P. Molero-Glez[1,2] & I. Melero[1,2,5,6,7,8,9]*

[1]Program of Immunology and Immunotherapy, Cima Universidad de Navarra, Pamplona; [2]Navarra Institute for Health Research (IDISNA), Pamplona, Spain; [3]Human Oncology and Pathogenesis Program (HOPP), Immuno-Oncology Service, Memorial Sloan Kettering Cancer Center, New York; [4]Parker Institute for Cancer Immunotherapy, New York, USA; [5]Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid; [6]Department of Immunology and Immunotherapy, Clínica Universidad de Navarra, Pamplona; [7]Department of Oncology, Clínica Universidad de Navarra, Madrid; [8]Centro del Cancer de la Universidad de Navarra (CCUN), Pamplona, Spain; [9]Nuffield Department of Medicine (NDM), University of Oxford, Oxford, UK

Available online 12 June 2024

Adoptive T-cell therapies (ACTs) including tumor-infiltrating lymphocytes and engineered T cells (transgenic T-cell receptor and chimeric antigen receptor T cells), have made an important impact in the field of cancer treatment over the past years. Most of these therapies are typically administered systemically in approaches that facilitate the elimination of hematologic malignancies. Therapeutical efficacy against solid tumors, however, with the exception of tumor-infiltrating lymphocytes against melanoma, remains limited due to several barriers preventing lymphocyte access to the tumor bed. Building upon the experience of regional administration in other immunotherapies, the regional administration of adoptive cell therapies is being assessed to overcome this challenge, granting a first round of access of the transferred T cells to the tumor niche and thereby ensuring their activation and expansion. Intralesional and intracavitary routes of delivery have been tested with promising antitumor objective responses in preclinical and clinical studies. Additionally, several strategies are being developed to further improve T-cell activity after reinfusing them back to the patient such as combinations with other immunotherapy agents or direct engineering of the transferred T cells, achieving long-term immune memory. Clinical trials testing different regional adoptive T-cell therapies are ongoing but some issues related to methodology of administration and correct selection of the target antigen to avoid on-target/off-tumor side-effects need to be further evaluated and improved. Herein, we discuss the current preclinical and clinical landscape of intratumoral and locoregional delivery of adoptive T-cell therapies.

Key words: regional, cell therapies, CAR, TIL, TCR

ADOPTIVE T-CELL THERAPIES IN THE CLINIC

The main current cancer immunotherapy strategies involve (i) empowering pre-existing antitumor T-cell responses through immune checkpoint blockade (ICB), (ii) generating *de novo* T-cell responses via vaccination, and (iii) introducing *ex-vivo cultured* tumor-specific T cells as an adoptive cell therapy. ICBs such as programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibitors, have shown unprecedented clinical benefit against multiple tumor types and are now a standard treatment option for a broad spectrum of malignant diseases.[1] Low response rates and progression after remission, however, represent unmet major challenges for ICB-based immunotherapies.[2] Vaccination strategies as well as ICBs, despite the presence of tumor-specific T cells in the patients' peripheral blood and tumor microenvironment,[3] are restricted by the T-cell fold expansion required to induce meaningful and long-lasting clinical activity.[4] This limitation is conditioned by the differentiation status of the tumor-specific T cells[5] under the influence of an immune-suppressive tumor microenvironment.[6] These limiting variables may in principle be overcome using adoptive T-cell transfer.

Adoptive T-cell therapies (ACTs) consist of the infusion of *ex vivo* expanded lymphocytes enabled with anticancer activity. The two main ACT modalities are based on the infusion in large numbers of naturally occurring autologous tumor-infiltrating lymphocytes (TILs)[3] and on the infusion of engineered T cells to specifically recognize tumor-associated

*Correspondence to: Dr Irene Olivera, Program of Immunology and Immunotherapy, Cima Universidad de Navarra, Avda. Pio XII, 55, 31008 Pamplona, Navarra, Spain. Tel: +34 948194700
E-mail: iolivera@alumni.unav.es (I. Olivera).
*Dr Ignacio Melero, Program of Immunology and Immunotherapy, Cima Universidad de Navarra, Avda. Pio XII, 55, 31008 Pamplona, Navarra, Spain. Tel: +34 948194700
E-mail: imelero@unav.es (I. Melero).

antigens (TAAs) by the genetic supply of an exogenous T-cell receptor (TCR) or a chimeric antigen receptor (CAR).[7,8]

Clinical trials testing the infusion of autologous TILs to treat metastatic melanoma patients have shown durable objective response rates.[9-11] The efficacy of TIL transfer in the treatment of other solid tumors, however, has been more limited. Different strategies are being evaluated to improve TIL therapeutic activity.[11] Advances in sequencing techniques have enabled the possibility of retrieving TCR sequences from TILs or peripheral blood lymphocytes with specificity to TAAs.[12,13] The low abundance of tumor-specific T cells within patient T-cell repertoires and the patient-specific nature of the epitopes expressed by tumors are the main limiting factors for the isolation and clinical scalability of therapeutic TCRs. Based on the phenotypic, functional or transcriptomic profile of tumor specific T-cell clones, multiple strategies are under development to identify and isolate tumor-specific TCRs from TILs, patient circulating T cells or healthy donor-derived T cells.[14] High-throughput functional tools to screen patient-derived TCR libraries are being implemented to identify and validate tumor-reactive T-cell receptors.[15,16] Additionally, single-cell transcriptomic signatures from TIL samples can be used to identify tumor-reactive TCR clonotypes. These tumor-specific T-cell subsets can be found in transcriptional clusters with up-regulated expression of chemokines[17] and exhaustion-related genes.[18,19]

The therapeutic potential of TCR-engineered T-cell therapies able to recognize neoantigens was shown in a case report study, showing strong antitumor responses in a patient with lung cancer treated with TILs enriched with T cells expressing a high-affinity TCR for KRAS G12D mutation.[20] All seven lung metastases regressed after treatment, but the tumor eventually progressed from a tumor lesion that lost the antigen-presenting HLA-C allele. Resistance mechanisms to TIL therapy include the loss of expression of the targeted antigens or antigen-presentation molecules due to immunological pressure,[21] the exhausted phenotype of TILs or the scanty T-cell recovery.[22] In order to overcome these challenges, T cells engineered with transgenic TAA-specific TCRs are being developed. For instance, autologous T cells collected from peripheral blood transduced with transgenic TCR targeting KRAS G12D induced regression of visceral metastasis in a patient with a metastatic pancreatic cancer.[23] Another proof-of-concept study demonstrated that p53 NeoAg-specific TCR-engineered T cells from peripheral blood had a less exhausted phenotype and a prolonged persistence compared with TILs naturally reactive to p53 mutations in human solid tumors.[24] In this study, a patient with chemorefractory breast cancer treated with these p53-TCR-transduced T cells had an objective tumor regression that lasted 6 months.[24] The generation of libraries of TCRs targeting shared neoantigens derived from common oncogenic driver mutations and screenings matching the antigen mutations with HLA alleles in cancer patients constitute an opportunity for personalized ACTs for each tumor type.[25]

Regarding CAR-based ACTs, their antitumor activity has been extensively demonstrated in hematological cancers. BCMA re-directed CAR-T cells for multiple myeloma and CD19-CAR-T cells for the treatment of acute B-cell lymphoblastic leukemia, and B-cell lymphomas are the only ACT-based treatments currently approved by the Food and Drug Administration (FDA) due to the remarkable clinical success.[26] However, toxicities associated with the CAR-T cell infusion such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) are frequently observed.[27] Beyond hematologic malignancies, CAR-T application for solid tumors has so far been unsuccessful. A phase I/II clinical trial using human epidermal growth factor receptor 2 (HER2)-CAR-T cells in patients with HER2-positive sarcoma reported complete responses only in 3 out of 16 treated patients.[28] A significant challenge in developing CAR-T cells for solid tumors is that many potential target antigens are also expressed on healthy tissues, resulting in a high risk of on-target, off-tumor toxicities.

A major limitation in the clinical development and therapeutic success of many of the immunotherapeutic approaches for solid tumors is the fact that tumors are shielded behind an immunosuppressive environment impeding efficient antitumor T-cell infiltration as well as local, expansion and persistence.[6] As an alternative to systemic delivery, growing data support that the regional/intratumoral administration of ACT may help overcome the spatiotemporal obstacles of the tumor microenvironment, minimize toxicity, and lead to a long-lasting systemic immunity. In this review, we provide an overview of the opportunities and challenges pertaining to the regional/intratumoral delivery of ACTs (Figure 1).

PRECLINICAL AND CLINICAL VALUE OF INTRATUMORAL AND LOCOREGIONAL ADMINISTRATION OF CANCER IMMUNOTHERAPIES

Most of the current immunotherapy approaches are infused systemically. Intravenous (i.v.) administration has several advantages such as easy access in all patients and the systemic bioavailability of the agent. However, while hematological malignancies stay within the peripheral compartment, solid tumors are commonly surrounded by physical barriers that avoid efficient delivery of those immunotherapy agents to the tumor lesion. In addition, i.v. delivery usually results in immune-related adverse events (irAEs) associated with systemic inflammation due to on-target/off tumor effects.[29-31] To overcome these limitations, intratumoral or locoregional administrations of several cancer immunotherapy agents are being evaluated in multiple preclinical and clinical studies involving ICB agents, oncolytic viruses, toll-like receptor (TLR) agonists or ACTs.

The clinical applicability of some ICB agents is limited due to irAEs.[30-32] Intratumor administration of immune checkpoint inhibitors has been evaluated as an alternative to i.v. delivery in order to mitigate toxicity and improve the dose/efficacy ratio. Preclinical data in mouse models demonstrate that intratumoral delivery of anti-CTLA-4 induces tumor

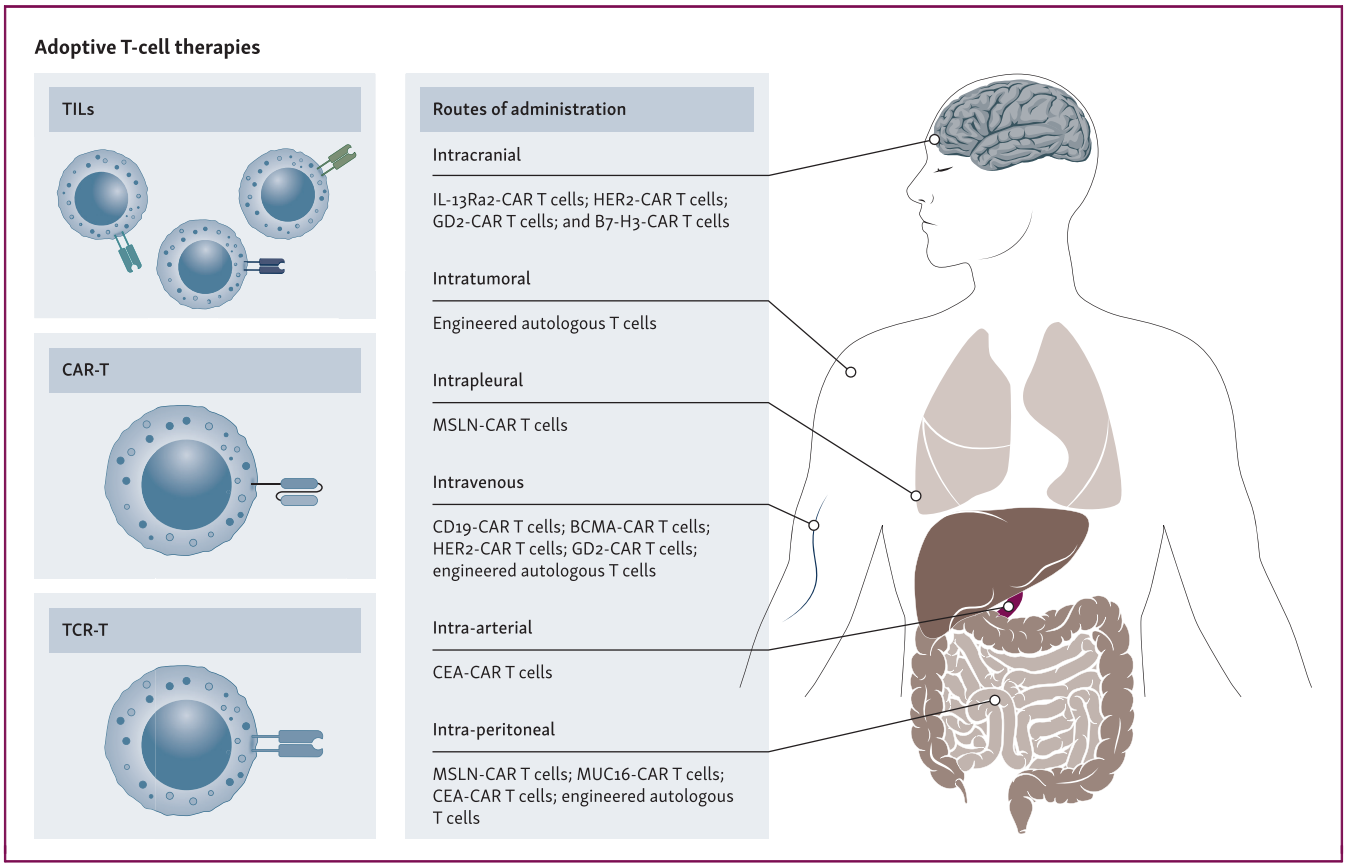

Figure 1. Routes of administration of adoptive T-cell therapies used for cancer treatment. A list of engineered T-cell therapies or TIL is provided along with their specific routes of administration, either systemically or locoregionally.
CAR, chimeric antigen receptor; CEA, carcinoembryonic antigen; HER2, human epidermal growth factor receptor 2; IL-13-Rα2, interleukin-13 receptor subunit alpha-2; MSLN, mesothelin; TCR, T-cell receptor; TILs, tumor-infiltrating lymphocytes.

eradication in a CD8- and cDC1-dependent manner while limiting the irAEs.[33] Following this rationale, intratumoral administration of TLR9 agonists to promote dendritic cell activation together with ipilimumab is being evaluated in patients with anti-PD-1 refractory cancer (NCT03445533). Additionally, a phase I clinical trial tested the perioperative intracranial administrations of ipilimumab and nivolumab plus i.v. administration of nivolumab in patients with recurrent glioblastoma (NCT03233152).[34] Another phase Ib study compared intratumoral versus i.v. ipilimumab with nivolumab in untreated metastatic melanoma patients (NCT02857569). Results showed reduced toxicity at 6 months with intratumoral (30%) compared with i.v. (57.1%) administration. Importantly, response rates were similar (50% in the intratumoral group and 65% in the i.v. group). Previously, the combination of intratumoral ipilimumab with interleukin 2 (IL-2) was also tested in clinical trials for patients with advanced melanoma. In this case, the reported overall response was 40% and it correlated with an enhanced immune response. In addition, the efficacy of regional delivery of other ICBs is also being tested such as anti-CD137 alone in combination with nivolumab (NCT03792724).

Oncolytic viruses are designed to selectively replicate in cancer cells and trigger immunogenic cell death that boosts an immune response against the tumor lesion. Intratumoral injection of T-VEC, a form of herpes simplex virus-1 modified to express granulocyte—macrophage colony-stimulating factor (GM-CSF), is already approved by the FDA and the European Medicines Agency for the treatment of superficial melanoma metastasis. In the phase III trial, T-VEC resulted in 47% of complete responses in the injected lesions and 22% in the non-injected lesions, indicating that intratumoral delivery can also induce systemic antitumor immunity.[35]

Additionally, stereotactic infusion of other strains of oncolytic viruses into brain tumors is being tested alone or in combination with ICB-based immunotherapies. Intratumor virotherapy for brain tumors was pioneered by a study using a poliovirus—rhinovirus chimeric vector in 60 patients with recurrent glioblastoma, reaching a plateau at 24 months of 21% at the cost of side-effects of local inflammation that could be mitigated with steroids.[36] The oncolytic adenovirus DNX-2401 alone induced a reduction of the tumor size in 9 out of 12 pediatric patients with diffuse pontine glioma.[37] In recurrent glioblastoma, 56.2% of patients who received DNX-2401 plus pembrolizumab exhibited an objective clinical response.[38]

To mimic the presence of microbial pathogens in the tumor tissue, TLR3, TLR7, TLR9, and STING agonists are being tested following intratumoral delivery. Evidence for

https://doi.org/10.1016/j.iotech.2024.100715

additive or synergistic effects with checkpoint inhibitors is often reported in mouse models and in some patients with a variety of solid tumors in early-phase clinical trials.[39] Regional administration of other immunotherapy approaches has been reviewed by others.[40]

PRECLINICAL EXPERIENCE OF INTRATUMORAL AND LOCOREGIONAL ADOPTIVE T-CELL TRANSFER

Stromal barriers and a poor vascular access together with an immunosuppressive tumor microenvironment are a hurdle for T-cell infiltration and activation, which are crucial for the antitumor efficiency of ACTs. The main rationale for regional delivery of ACT is granting access of the infused T cells to the tumor niche not only to eliminate the tumor, but also to ensure early activation and expansion as a result of immediate antigen encounter. In contrast to systemically administered T cells, which may face limitations regarding tumor migration and infiltration due to the absence of an attractive tumor chemokine gradient, regionally delivered T cells encounter cognate antigens and become activated and proliferate. This leads to increased local chemokine expression and enhanced circulation for effective infiltration of distant tumor sites. Indeed, cytokines and chemokines produced by T cells in the tumor microenvironment can positively influence the immunogenicity of local tumor immune environment, boosting the activation of native non-infused immune cells. The benefits of the intratumor T-cell delivery have been demonstrated in multiple orthotopic solid tumor models. In a pleural mesothelioma tumor model, while intravenously administered CAR-T got retained in the lungs, regional intrapleural injection enhanced penetration into the tumors, activation and expansion of T cells. As a result, tumor eradication occurred even with low doses of CAR-T cells.[41] Moreover, a rapid acquisition of an effector phenotype of T cells induces the release of several soluble mediators to the tumor microenvironment,[42,43] facilitating tumor elimination and the reprogramming of the endogenous immune compartment.[43] Systemic immune responses in non-treated lesions were also observed in several preclinical studies after intratumor T-cell delivery.[44-46] Interestingly, rechallenged cured mice were also able to control both antigen-positive tumor growth and antigen-negative tumors after locoregional adoptive transfer of B7-H3-specific CAR-T cells,[47] suggesting that regional ACT may induce antigen-spreading and long-term systemic immune responses against cancer recurrence.

A limiting factor in the clinical development of ACT is the toxicity associated with on-target/off-tumor expression of the targeted Ag. In the case of CAR-T cells, the main and most common side-effects are the generation of CRS and ICANS caused by a strong overactivation of T cells, resulting in the expression of high amounts of cytokines including IL-6, IL-1b, GM-CSF or tumor necrosis factor- α (TNF-α) among others.[29] Despite the existence of therapeutic interventions to treat ICANS and CRS in the clinic, locoregional delivery of ACTs has been shown to reduce systemic levels of several proinflammatory cytokines.[47]

Regional approaches to deliver ACTs for treatment of several tumor types are under preclinical evaluation. These strategies include intraventricular delivery for brain tumors, intraperitoneal administration for peritoneal carcinomatosis or intratumoral for other solid tumors such as melanoma lesions. Intratumoral administration of mouse and human TILs delayed tumor growth in different syngeneic and xenografted tumor mouse models.[45,46] Interestingly, such results were improved after T-cell engineering with messenger RNA (mRNA) encoding different proinflammatory cytokines such as IL-12, CD137L,[45] and IL-18,[46] showing excellent antitumor responses even in distant non-treated tumor lesions. These data were confirmed with TILs, transgenic TCR, and CAR-T cells. Therapeutic efficacy and safety of the locoregional administration of autologous tumor-reactive T cells is under evaluation in clinical trials in patients with cervical cancer (NCT03362619), but the efficacy seems to be limited to tumors with high mutational burdens.

Regarding brain tumors, high intracranial pressure and the presence of the blood brain barrier limit the infiltration of T cells within the tumor bed[48] which means that regional delivery of ACTs makes special sense for treatment of brain tumors.

B7-H3 (CD276) is member of the B7 family with immunoregulatory roles that is highly expressed in high-grade glioma, medulloblastoma, atypical teratoid/rhabdoid tumor, and diffuse intrinsic pontine glioma.[49] Preclinical results showed that B7-H3 CAR-T cells delivered intratumorally or alternatively, as sometimes intratumoral administration in the brain is not possible, intracerebroventricular delivery, effectively eradicated the tumor lesions and limited the systemic release of proinflammatory cytokines, whereas intravenously injected CAR-T cells did not,[47] suggesting at least in this case the advantages of locoregional administration of ACT for treatment of brain tumors compared with the systemic route.

Epidermal growth factor receptor (EGFR) variant III (EGFRvIII) is the most common variant of EGFR, expressed in ~30% of glioblastoma patients and involved in the tumorigenic phenotype.[50] I.V. injection of EGFRvIII CAR-T cells showed limited efficacy, although antigen decrease was observed in the majority of patients. An increase in regulatory T cells (Tregs) and antigen escape may explain this clinical outcome. Preclinical studies involving intracerebroventricular infusion of EGFRvIII-CAR-T cells showed a potent antitumor response in mouse models of glioblastoma without detectable toxicities.[51] This approach was even able to circumvent antigen loss[51] and redirect T cells[52] by means of the paracrine secretion of a bispecific T-cell engager (BiTE) against wild-type EGFR, thereby explaining the improvement in therapeutic effects.

Other studies tested intracavitary adoptive transfer of T cells for the treatment of peritoneal malignancies such as ovarian cancer and mesothelioma. The pattern of spread of these types of cancers over a serosa surface may lend them to regional delivery. Intracavitary delivery of engineered TCR-transgenic[53] and CAR-T cells[44]

Table 1. Clinical trials conducted or ongoing using regional/intratumoral administration of adoptive T-cell therapies for cancer treatment

Delivery route	ACT type	Phase	Cancer	Status	Trial number (ref.)
Intratumoral, intraperitoneal or intravenous	Engineered autologous T lymphocytes reactive to human papillomavirus antigens	I/II	Cervical cancer	Unknown	NCT03362619
Intracranial	IL-13Ra2-CAR T + aldesleukin	I	Recurrent glioblastoma (rGBM)	Completed	NCT01082926[54]
Intracranial	IL13Ra2-CAR T +/− ipilimumab and nivolumab	I	rGBM	Recruiting	NCT04003649
Intracavitary/intratumoral and intracerebroventricular	HER2-CAR T + truncated CD19	I	Recurrent or refractory grade III-IV glioma	Active, not recruiting	NCT03389230[57]
Repeated intracerebroventricular	B7-H3 CAR-T cells	I	Recurrent brain tumors and diffuse intrinsic pontine glioma (DIPG)	Recruiting	NCT04185038[56]
Intracerebroventricular	EGFRvIII CAR-T cells secreting BiTE against EGFR	I	Recurrent glioblastoma	Recruiting	NCT05660369[59]
Intravenous + intracerebroventricular	GD2-CAR-T cells	I	DIPG and spinal diffuse midline glioma	Recruiting	NCT04196413[60]
Intracranial	HER2-CAR with truncated EGFR	I	HER2-positive recurrent/ refractory pediatric CNS tumors	Active, not recruiting	NCT03500991[62]
Intrapleural	Mesothelin (MSLN)-CAR T	I/II	Malignant pleural disease	Active, not recruiting	NCT02414269
Intrapleural	MSLN-CAR-T cells + PD-1 dominant negative	I	Malignant pleural disease	Recruiting	NCT04577326
Intraperitoneal	MCY-M11 (MSLN-CAR-T cells)	I	Advanced ovarian cancer and peritoneal mesothelioma	Terminated	NCT03608618[63]
Intraperitoneal	CEA-CAR-T cells	I	CEA-positive adenocarcinoma peritoneal metastases or malignant ascites	Withdrawn	NCT03682744
Intravenous and intraperitoneal	IL-12-secreting MUC16ecto-CAR-T cells	I	Recurrent Muc16ecto-positive solid tumors	Active, not recruiting	NCT02498912[64]
Intraperitoneal	MUC16-CAR T + membrane-bound IL-15	I/Ib	Platinum-resistant ovarian cancer	Recruiting	NCT03907527[78]
Intra-arterial	CEA-CAR-T cells	I	CEA-positive liver metastases	Completed	NCT01373047[68]
Intra-arterial	CEA-CAR-T cells + selective internal radiation therapy	Ib	CEA-positive liver metastases	Completed	NCT02416466[69]
Intra-arterial	CEA-CAR-T cells + chemotherapy versus chemotherapy alone	IIb	CEA-positive pancreatic cancer and liver metastases	Withdrawn	NCT04037241

ACT, adoptive T-cell therapy; BiTE, bispecific T-cell engager; CEA, carcinoembryonic antigen; CAR, chimeric antigen receptor; CNS, central nervous system; DIPG, diffuse intrinsic pontine glioma; EGFRvIII, epidermal growth factor receptor variant III; HER2, human epidermal growth factor receptor 2; IL, interleukin; IL-13-Ra2, interleukin-13 receptor subunit alpha-2; MSLN, mesothelin; PD-1, programmed cell death protein 1; rGBM, recurrent glioblastoma.

promoted eradication of peritoneal metastases in ovarian tumor mouse models. An enhanced T-cell persistence after intracavitary delivery compared with the i.v. counterpart may explain the improvement of the antitumor responses.

CLINICAL STUDIES INCORPORATING LOCAL AND REGIONAL T-CELL TRANSFER FOR GLIOBLASTOMA HEPATOCELLULAR CARCINOMA AND MESOTHELIOMA

As therapeutic efficacy of TILs is generally limited to tumors with a high mutational burden such as melanoma, main clinical efforts to apply regional T-cell transfer in the clinic are currently focused on CAR-T cell therapies (Table 1). IL13Rα2 is a high-affinity IL-13 receptor, overexpressed on >60% of glioblastoma tumors but not in healthy brain tissue. In fact, high expression of IL13Rα2 has been associated with a mesenchymal subtype of glioblastoma with poor patient prognosis.[55] These data provided the rationale for generating IL13Rα2-specific CAR-T cells. The safety and preliminary efficacy of intracranial infusion of IL13Rα2-CAR-T cells in combination with IL-2 (aldesleukin) was tested in a phase I clinical trial for treatment of recurrent glioblastoma. These CAR-T cells were genetically engineered to maintain their effector functions even in the presence of dexamethasone that is commonly used to both mitigate tumor-associated neuroedema and avoid the rejection of allogeneic cells. Preliminary evidence of antitumor responses was observed in four out of the six treated patients together with a good tolerability profile.[54] Currently, the combination of IL13Rα2-CAR-T cells with ipilimumab and nivolumab is under clinical evaluation (NCT04003649) to further improve the therapeutic efficacy. The intratumoral and intracavitary delivery of memory HER2-specific CAR-T cells for treatment of refractory glioma is also being tested in clinical trials (NCT03389230).

The bioactivity and safety of repeated intraventricular administration of B7-H3 CAR-T cells was tested in a phase I clinical trial for treatment of recurrent brain tumors and diffuse intrinsic pontine glioma (NCT04185038).[56] Three patients were treated with 40 repeated injections of CAR-T cells with no dose-limiting toxicities. Despite the small treated cohort, a promising clinical response was observed in one patient after 12 months. Importantly, this patient showed evidence of local immune activation. Similar results were previously reported with HER2-specific CAR-T cells in the BrainChild-01 trial (NCT03500991).[57]

Increased infiltration of Tregs and antigen loss after i.v. injection of CAR-T cells seems to mediate adaptive resistance in patients, thereby limiting the efficacy of ACTs.[58] In this regard, preclinical studies developed EGFRvIII-CAR-T cells engineered to secrete a BiTE against wild-type EGFR to both redirect Tregs[52] that are EGFR+, and circumvent antigen escape.[51] A single intracerebroventricular injection of these EGFRvIII CAR-T cells demonstrated rapid radiographic tumor regressions in three patients with recurrent glioblastoma without the observation of any dose-limiting toxicities. Nevertheless, the clinical responses were transient in two out of the three patients.[59] Intracerebroventricular infusion of GD2-CAR-T cells was also tested in four patients with H3K27M-mutated diffuse intrinsic pontine glioma following i.v. administration of the same CAR (NCT04196413). This first-in-human phase I clinical trial reported objective clinical and radiographic responses in three out of four pediatric patients. The results correlated with higher cytokine concentration, better CAR-T cell persistence, and lower infiltration of Tregs in the cerebrospinal fluid after intracerebroventricular administration compared with the i.v. route,[60] suggesting the advantages of locoregional administration of ACTs for treatment of brain tumors compared with the systemic route.

Intraperitoneal and intrapleural delivery of ACTs are being tested for treatment of malignant pleural diseases and intracavitary malignancies such as ovarian cancer, peritoneal mesothelioma, pancreatic cancer, and peritoneal metastasis. Mesothelin (MSLN) is a TAA highly expressed in 64%-90% of the epithelioid subtype of malignant mesothelioma[61] whereas there is almost no expression in healthy tissues. Intrapleural injection of MSLN-specific CAR-T cells induced partial responses in 8 out of 20 treated patients that remained up to 6 months (NCT02414269).[62] Additionally, an ongoing clinical trial is evaluating the intraperitoneal administration of transiently engineered T cells with an anti-MSLN CAR (MCY-M11) for the treatment of ovarian cancer and peritoneal mesothelioma (NCT03608618). Preliminary data presented in ASCO 2020 showed that MCY-M11 CAR therapy was safe, and three patients achieved stable disease for at least 2 months.[63] Other trials exploring locoregional infusion of ACTs include carcinoembryonic antigen (CEA)-specific CAR-T cells for adenocarcinoma peritoneal metastases (NCT03682744) or MUC16-CAR T for ovarian cancer (NCT02498912).[64]

Intra-arterial administration is another route of, to some extent, regional delivery that is being used for the treatment of hepatocellular carcinoma or liver metastasis. Hepatic artery infusion via a catheter has been previously tested for chemotherapy and it is feasible.[65] This route of delivery preferentially targets liver cancer because these tumor lesions are normally perfused by the hepatic artery while the healthy hepatocytes are normally perfused by the portal circulation.[66] Although no improvement of intra-arterial infusion was observed in preclinical studies compared with i.v. administration,[67] the safety and efficacy of intra-arterial delivery is being evaluated in the clinical setting. A phase I trial demonstrated that repeated intra-arterial infusion of CEA-specific CAR-T cells for the treatment of CEA-positive liver metastasis is safe, but only stable disease in one out of six patients was the best response.[68] In order to improve efficacy, intra-arterial administration of CEA CAR-T cells was tested in combination with selective internal radiation therapy. With this setting, one out of six patients achieved a complete liver metabolic response assessed by positron emission tomography (PET) along with a concomitant reduction of serum CEA level. However, the responding patient eventually died due to a pancreatic tumor progression.[69]

THERAPEUTIC APPROACHES TO FURTHER ENHANCE EFFICACY

After T-cell reinfusion to the patient, transferred T cells have to overcome a number of obstacles in the tumor bed such as the presence of co-inhibitory signals or poor co-stimulation signaling that induce T-cell dysfunction. Immune checkpoint inhibitors in combination with ACTs have the potential to avoid T-cell exhaustion, improving their antitumor efficacy. For instance, a phase I clinical trial tested whether the blockade of PD-1/PD-L1 axis was able to augment the therapeutic efficacy of the regional administration of MSLN-CAR-T cell efficacy in a phase I clinical trial (NCT02414269). The median overall survival from CAR-T cell infusion was 23.9 months (1-year overall survival, 83%) and 8 out of 18 patients exhibited stable disease, with a complete metabolic response on PET scan in 2 of them.[62] The finding that CAR-T cells were detected, persisting in peripheral blood after 100 days in ∼40% of patients, highlights the potential to attain long-term antitumor immunity. Another strategy to modulate the co-stimulation signal is to directly engineer the T cells. For instance, Dr Marcela Maus' group knocked down PD-1, the endogenous TCR and beta-2 microglobulin with CRISPR-Cas9 to create 'universal' EGFRvIII CAR-T cells for a preclinical model of glioblastoma. Interestingly, the PD-1 knockout (KO)-engineered EGFRvIII CAR-T cells exerted an improved antitumor response that prolonged the survival of mice after intraventricular injection in the brain, but not following i.v. administration.[70] Safety of i.v. infusion of PD-1 KO engineered CAR-T cells was demonstrated in patients with non-small-cell lung cancer,[71] MSLN-positive solid tumors[72] and esophageal cancer (NCT03706326),[73] but the therapeutic efficacy of the regional infusion of these modified CAR-T cells remains unknown. Moreover, the safety of intrapleural administration of MSLN-CAR-T cells engineered with a PD-1 dominant negative receptor is under clinical evaluation for malignant pleural mesothelioma (NCT04577326).

Another limitation for the activation and expansion of the transferred T cells is the poor immune-stimulatory cytokine support within the tumor microenvironment. To overcome this limitation, genetic engineering of T cells to express different proinflammatory cytokines is being evaluated in preclinical and clinical studies. IL-18 and IL-12 are immune-stimulatory cytokines mainly secreted by myeloid cells that

lead to IFN-γ secretion by T cells and direct control of the tumor growth by reshaping all the immune compartment within the tumor microenvironment. Constitutive secretion of IL-18 by CAR-T cells improved T-cell expansion, leading to superior antitumor responses in mouse models of small-cell lung cancer,[74] acute lymphoblastic leukemia, and melanoma.[75] Indeed, these results led to conducting a phase I clinical trial in patients with CD19-positive relapsed/refractory non-Hodgkin's lymphoma or chronic lymphocytic leukemia who had received at least two lines of therapy. Preliminary data presented in ASCO 2022 by Dr Carl June is promising, with four out of seven complete responses and three partial responses (NCT04684563).[76]

Regarding IL-12, despite its potent antitumor effects in preclinical studies, constitutive secretion is not an option due to the appearance of toxic side-effects.[77] To limit IL-12 secretion to the tumor bed, several approaches have been developed to confine IL-12 expression to tumor-specific T cells. For instance, locoregional administration of engineered CAR-T cells with a membrane-bound IL-12 effectively exerted a superior tumor growth in several preclinical mouse models of peritoneal metastasis.[44] Alternatively, electroporation of T cells with IL-12 mRNA confers the possibility to transiently express the cytokine, limiting the associated toxicities. This approach induced rejection of both treated and distant non-injected tumors after repetitive intratumoral delivery of TILs and TCR-transgenic T cells in melanoma mouse models.[45] Similar antitumor effects were reported with intracavitary infusion of IL-12-engineered OT-I T cells to treat peritoneal metastasis.[53] A phase I clinical trial evaluated the feasibility and efficacy of intraperitoneal administration of IL-12-engineered MUC16-CAR-T cells in patients with recurrent high-grade serous ovarian cancer (NCT02498912). Although this therapeutic approach was well tolerated, the best response was stable disease.[64] Another clinical trial is testing MUC16-CAR-T cells with a membrane bound IL-15 for treatment of platinum-resistant ovarian cancer (NCT03907527), reporting a preliminary response rate of 20%.[78] The clinical benefit was limited, indicating that combining adoptive T-cell transfer with other immunotherapy approaches such as ICBs is needed to foster T-cell functions against solid tumors.

In order to improve the efficacy of ACTs with mRNA encoding IL-12 in mouse models, T cells were electroporated with either IL-12 or a decoy-resistant mutant form of IL-18 mRNA. The rationale for generating this variant of IL-18 is that it fails to bind to a decoy receptor called IL-18 binding protein (IL-18BP), which strongly limits the therapeutic potential of IL-18. Mixed populations of T cells engineered with either the non-repressive form of IL-18 or IL-12 exert a superior antitumor response, depending on changes in the glycosylation of T-cell surface proteins, miR-155 expression, and improvements in T-cell metabolism.[46] Interestingly, the therapeutic efficacy of this new approach was tested in cultures of mouse TILs, TCR-transgenic and gp75-specific CAR-T cells in melanoma and colon carcinoma mouse models. Whether these combinations will improve the therapeutic activity of intratumoral ACT in cancer patients remains unknown.

CONCLUDING REMARKS

Intratumoral or regional administration presents an opportunity to enhance the therapeutic activity of ACTs while mitigating the occurrence of systemic adverse events. Local T-cell therapy is becoming more accessible due to advancements in interventional radiology, enabling accurate delivery and improved outcomes. Standardized approaches, image-guided interventions, and novel biomaterials for sustained release can enhance intratumoral immunotherapy. The standardization of the methodology, such as the material of injection or the injection technique, is crucial to ensure large-scale extrapolation of clinical trials.[79]

Compiling preclinical and clinical evidence supports that locally transferred T cells can establish systemic immunity while reducing toxicity. Locally delivered T cells gain direct access to the tumor microenvironment, promoting their activation and proliferation. This is crucial since cognate antigen is immediately met while this is not the case upon i.v. delivery. In a way, this mechanism is expected to circumvent the obstacle of poor tissue penetration. Engineering T cells with transient expression of IL-12 and IL-18 induced long-term immune responses as a result of activating the endogenous immune system[45,46] and favoring epitope spreading in tumor mouse models.[45] Additionally, the safety of intra-arterial infusion of CEA-specific CAR-T cells plus selective internal radiation therapy was confirmed in a phase Ib clinical trial for CEA-positive liver metastases.[69] Evaluation of the efficacy of different combinations of ACTs with other immunotherapy approaches or traditional cancer treatments needs to be carried out in future preclinical and clinical studies. In summary, delivering ACTs locally or regionally will be particularly beneficial for treating localized tumors, leading to improved clinical outcomes and tolerability. Importantly, regional ACT can be combined with other immunotherapeutic approaches to ensure optimal long-term immune responses. By analogy, this is like deploying paratroopers behind enemy lines. So perhaps strategies with part of the ACT dose given locally and part systemically may be the best option.

FUNDING

This wok was supported by MINECO PID2020-112892RB-100 and PID2020-113174-RA-100 (MCIN/AEI/10.13039/501100011033) and by Instituto de Salud Carlos III (PI19/01128) co-financed by Fondos FEDER 'A way to make Europe', by the I-ON network supported by Bristol Myers Squibb to IM. This project has received funding from the European Union's Horizon 2020 research and innovation program [grant number 945393-T2EVOLVE] and Marie-Sklodowska-Curie [grant number 765394], by Cancer Research UK [grant number C18915/A29362], FCAECC and AIRC under the Accelerator Award Programme, Marató TV3 [grant number 488/C/2019], Fundación Fero (no grant number), Fundación La Caixa HR21-00083 Gobierno de Navarra Salud GN 39-2021, Gobierno de Navarra Proyecto LINTERNA Ref: 0011-1411-2020-000075, Mark Foundation (no grant number), Fundación BBVA (LEO23-2-9672) and Fundación Olga Torres (no grant number).

DISCLOSURE

IM has served as a consultant for Consultancy BMS, Roche, Genmab, Numab, Pieris Catalym, FSTAR, Third Rock, Amunix, Gossamer, Alligator, AstraZeneca, Pharmamar and has received grants from Roche, AstraZeneca, Bristol Myers, and Genmab. All other authors have declared no conflicts of interest.

REFERENCES

1. Sharma P, Siddiqui BA, Anandhan S, et al. The next decade of immune checkpoint therapy. *Cancer Discov.* 2021;11:838-857.
2. Schadendorf D, Hodi FS, Robert C, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. *J Clin Oncol*. 2015;33:1889-1894.
3. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science*. 2015;348:62-68.
4. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell*. 2017;168:707-723.
5. Koyama S, Akbay EA, Li YY, et al. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. *Nat Commun*. 2016;7:10501.
6. Olivera I, Luri-Rey C, Teijeira A, et al. Facts and hopes on neutralization of protumor inflammatory mediators in cancer immunotherapy. *Clin Cancer Res.* 2023;29:4711-4727.
7. Finck AV, Blanchard T, Roselle CP, Golinelli G, June CH. Engineered cellular immunotherapies in cancer and beyond. *Nat Med*. 2022;28:678-689.
8. Chandran SS, Klebanoff CA. T cell receptor-based cancer immunotherapy: emerging efficacy and pathways of resistance. *Immunol Rev.* 2019;290:127-147.
9. Rohaan MW, Borch TH, van den Berg JH, et al. Tumor-infiltrating lymphocyte therapy or ipilimumab in advanced melanoma. *N Engl J Med.* 2022;387:2113-2125.
10. van den Berg JH, Heemskerk B, et al. Tumor infiltrating lymphocytes (TIL) therapy in metastatic melanoma: boosting of neoantigen-specific T cell reactivity and long-term follow-up. *J Immunother Cancer.* 2020;8:e000848.
11. Klobuch S, Seijkens TTP, Schumacher TN, Haanen JBAG. Tumour-infiltrating lymphocyte therapy for patients with advanced-stage melanoma. *Nat Rev Clin Oncol*. 2024;21:173-184.
12. Chandran SS, Ma J, Klatt MG, et al. Immunogenicity and therapeutic targeting of a public neoantigen derived from mutated PIK3CA. *Nat Med*. 2022;28:946-957.
13. Malekzadeh P, Pasetto A, Robbins PF, et al. Neoantigen screening identifies broad TP53 mutant immunogenicity in patients with epithelial cancers. *J Clin Invest*. 2021;129:1109-1114.
14. Klebanoff CA, Chandran SS, Baker BM, Quezada SA, Ribas A. T cell receptor therapeutics: immunological targeting of the intracellular cancer proteome. *Nat Rev Drug Discov.* 2023;22:996-1017.
15. Moravec Z, Zhao Y, Voogd R, et al. Discovery of tumor-reactive T cell receptors by massively parallel library synthesis and screening. *Nat Biotechnol*. 2024. https://doi.org/10.1038/s41587-024-02210-6.
16. Cattaneo CM, Battaglia T, Urbanus J, et al. Identification of patient-specific CD4+ and CD8+ T cell neoantigens through HLA-unbiased genetic screens. *Nat Biotechnol*. 2023;41:783-787.
17. Hanada K-I, Zhao C, Gil-Hoyos R, et al. A phenotypic signature that identifies neoantigen-reactive T cells in fresh human lung cancers. *Cancer Cell*. 2022;40:479-493.e6.
18. Lowery FJ, Krishna S, Yossef R, et al. Molecular signatures of antitumor neoantigen-reactive T cells from metastatic human cancers. *Science*. 2022;375:877-884.
19. Komuro H, Shinohara S, Fukushima Y, et al. Single-cell sequencing on $CD8^+$ TILs revealed the nature of exhausted T cells recognizing neoantigen and cancer/testis antigen in non-small cell lung cancer. *J Immunother Cancer.* 2023;11:e007180.
20. Tran E, Robbins PF, Lu YC, et al. T-cell transfer therapy targeting mutant KRAS in cancer. *N Engl J Med*. 2016;375:2255-2262.
21. Rodems TS, Heninger E, Stahlfeld CN, et al. Reversible epigenetic alterations regulate class I HLA loss in prostate cancer. *Commun Biol*. 2022;5:897.
22. Radvanyi LG, Bernatchez C, Zhang M, et al. Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. *Clin Cancer Res.* 2012;18:6758-6770.
23. Leidner R, Sanjuan Silva N, Huang H, et al. Neoantigen T-cell receptor gene therapy in pancreatic cancer. *N Engl J Med*. 2022;386:2112-2119.
24. Kim SP, Vale NR, Zacharakis N, et al. Adoptive cellular therapy with autologous tumor-infiltrating lymphocytes and T-cell receptor—engineered T cells targeting common p53 neoantigens in human solid tumors. *Cancer Immunol Res*. 2022;10:932-946.
25. Olivera I, Etxeberria I, Bolaños E, Gato-Cañas M, Melero I. Exploiting TCR recognition of shared hotspot oncogene-encoded neoantigens. *Clin Cancer Res.* 2020;26:1203-1204.
26. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat Rev Clin Oncol*. 2023;20:359-371.
27. Schubert ML, Schmitt M, Wang L, et al. Side-effect management of chimeric antigen receptor (CAR) T-cell therapy. *Ann Oncol*. 2021;32:34-48.
28. Ahmed N, Brawley VS, Hegde M, et al. Human epidermal growth factor receptor 2 (HER2) — specific chimeric antigen receptor—modified T cells for the immunotherapy of HER2-positive sarcoma. *J Clin Oncol*. 2015;33:1688-1696.
29. Morris EC, Neelapu SS, Giavridis T, Sadelain M. Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. *Nat Rev Immunol*. 2022;22:85-96.
30. Zhou Y, Medik YB, Patel B, et al. Intestinal toxicity to CTLA-4 blockade driven by IL-6 and myeloid infiltration. *J Exp Med*. 2023;220:e20221333.
31. Perez-Ruiz E, Minute L, Otano I, et al. Prophylactic TNF blockade uncouples efficacy and toxicity in dual CTLA-4 and PD-1 immunotherapy. *Nature*. 2019;569:428-432.
32. Lozano AX, Chaudhuri AA, Nene A, et al. T cell characteristics associated with toxicity to immune checkpoint blockade in patients with melanoma. *Nat Med*. 2022;28:353-362.
33. Gilardi M, Saddawi-Konefka R, Wu VH, et al. Microneedle-mediated intratumoral delivery of anti-CTLA-4 promotes cDC1-dependent eradication of oral squamous cell carcinoma with limited irAEs. *Mol Cancer Ther.* 2022;21:616-624.
34. Duerinck J, Schwarze JK, Awada G, et al. Intracerebral administration of CTLA-4 and PD-1 immune checkpoint blocking monoclonal antibodies in patients with recurrent glioblastoma: a phase I clinical trial. *J Immunother Cancer.* 2021;9:e002296.
35. Andtbacka RHI, Ross M, Puzanov I, et al. Patterns of clinical response with talimogene laherparepvec (T-VEC) in patients with melanoma treated in the OPTiM phase III clinical trial. *Ann Surg Oncol*. 2016;23:4169-4177.
36. Desjardins A, Gromeier M, Herndon JE, et al. Recurrent glioblastoma treated with recombinant poliovirus. *N Engl J Med*. 2018;379:150-161.
37. Gállego Pérez-Larraya J, Garcia-Moure M, et al. Oncolytic DNX-2401 virus for pediatric diffuse intrinsic pontine glioma. *N Engl J Med*. 2022;386:2471-2481.
38. Nassiri F, Patil V, Yefet LS, et al. Oncolytic DNX-2401 virotherapy plus pembrolizumab in recurrent glioblastoma: a phase 1/2 trial. *Nat Med*. 2023;29:1370-1378.
39. Salas-Benito D, Pérez-Gracia JL, Ponz-Sarvisé M, et al. Paradigms on immunotherapy combinations with chemotherapy. *Cancer Discov.* 2021;11:1353-1367.
40. Melero I, Castanon E, Alvarez M, Champiat S, Marabelle A. Intratumoural administration and tumour tissue targeting of cancer immunotherapies. *Nat Rev Clin Oncol*. 2021;18:558-576.
41. Adusumilli PS, Cherkassky L, Villena-Vargas J, et al. Regional delivery of mesothelin-targeted CAR T cell therapy generates potent and long-lasting CD4-dependent tumor immunity. *Sci Transl Med*. 2014;6:261ra151.

42. Brown CE, Alizadeh D, Starr R, et al. Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *N Engl J Med*. 2016;375:2561-2569.
43. Pituch KC, Miska J, Krenciute G, et al. Adoptive transfer of IL13Rα2-specific chimeric antigen receptor T cells creates a pro-inflammatory environment in glioblastoma. *Mol Ther.* 2018;26:986-995.
44. Lee EHJ, Murad JP, Christian L, et al. Antigen-dependent IL-12 signaling in CAR T cells promotes regional to systemic disease targeting. *Nat Commun*. 2023;14:4737.
45. Etxeberria I, Bolaños E, Quetglas JI, et al. Intratumor adoptive transfer of IL-12 mRNA transiently engineered antitumor CD8+ T cells. *Cancer Cell*. 2019;36:613-629.e7.
46. Olivera I, Bolaños E, Gonzalez-Gomariz J, et al. mRNAs encoding IL-12 and a decoy-resistant variant of IL-18 synergize to engineer T cells for efficacious intratumoral adoptive immunotherapy. *Cell Rep Med*. 2023;4:100978.
47. Theruvath J, Sotillo E, Mount CW, et al. Locoregionally administered B7-H3-targeted CAR T cells for treatment of atypical teratoid/rhabdoid tumors. *Nat Med*. 2020;26:712-719.
48. Mapunda JA, Tibar H, Regragui W, Engelhardt B. How does the immune system enter the brain? *Front Immunol*. 2022;13:805657.
49. Maachani UB, Tosi U, Pisapia DJ, et al. B7—H3 as a prognostic biomarker and therapeutic target in pediatric central nervous system tumors. *Transl Oncol*. 2020;13:365-371.
50. Padfield E, Ellis HP, Kurian KM. Current therapeutic advances targeting EGFR and EGFRvIII in glioblastoma. *Front Oncol*. 2015;5:5.
51. Choi BD, Yu X, Castano AP, et al. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. *Nat Biotechnol*. 2019;37:1049-1058.
52. Choi BD, Gedeon PC, Herndon JEII, et al. Human regulatory T cells kill tumor cells through granzyme-dependent cytotoxicity upon retargeting with a bispecific antibody. *Cancer Immunol Res*. 2013;1:163-167.
53. Di Trani CA, Cirella A, Arrizabalaga L, et al. Intracavitary adoptive transfer of IL-12 mRNA-engineered tumor-specific $CD8^+$ T cells eradicates peritoneal metastases in mouse models. *Oncoimmunology.* 2023;12:2147317.
54. Brown CE, Rodriguez A, Palmer J, et al. Off-the-shelf, steroid-resistant, IL13Rα2-specific CAR T cells for treatment of glioblastoma. *Neuro Oncol*. 2022;24:1318-1330.
55. Brown CE, Warden CD, Starr R, et al. Glioma IL13Rα2 is associated with mesenchymal signature gene expression and poor patient prognosis. *PLoS One*. 2013;8:e77769.
56. Vitanza NA, Wilson AL, Huang W, et al. Intraventricular B7-H3 CAR T cells for diffuse intrinsic pontine glioma: preliminary first-in-human bioactivity and safety. *Cancer Discov.* 2023;13:114-131.
57. Vitanza NA, Johnson AJ, Wilson AL, et al. Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: an interim analysis. *Nat Med*. 2021;27:1544-1552.
58. O'Rourke DM, Nasrallah MP, Desai A, et al. A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci Transl Med*. 2017;9:eaaa0984.
59. Choi BD, Gerstner ER, Frigault MJ, et al. Intraventricular CARv3-TEAM-E T cells in recurrent glioblastoma. *N Engl J Med*. 2024;390:1290-1298.
60. Majzner RG, Ramakrishna S, Yeom KW, et al. GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas. *Nature*. 2022;603:934-941.
61. Chu GJ, Linton A, Kao S, et al. High mesothelin expression by immunohistochemistry predicts improved survival in pleural mesothelioma. *Histopathology.* 2023;83:202-210.
62. Adusumilli PS, Zauderer MG, Rivière I, et al. A phase I trial of regional mesothelin-targeted CAR T-cell therapy in patients with malignant pleural disease, in combination with the anti—PD-1 agent pembrolizumab. *Cancer Discov.* 2021;11:2748-2763.
63. Annunziata CM, Ghobadi A, Pennella EJ, et al. Feasibility and preliminary safety and efficacy of first-in-human intraperitoneal delivery of MCY-M11, anti-human-mesothelin CAR mRNA transfected into peripheral blood mononuclear cells, for ovarian cancer and malignant peritoneal mesothelioma. *J Clin Oncol*. 2020;38:3014.
64. O'Cearbhaill RE, Park JH, Halton EF, et al. A phase I clinical trial of autologous chimeric antigen receptor (CAR) T cells genetically engineered to secrete IL-12 and to target the MUC16ecto antigen in patients (pts) with MUC16ecto+ recurrent high-grade serous ovarian cancer (HGSOC). *Gynecol Oncol*. 2020;159:42.
65. Kemeny NE, Melendez FDH, Capanu M, et al. Conversion to resectability using hepatic artery infusion plus systemic chemotherapy for the treatment of unresectable liver metastases from colorectal carcinoma. *J Clin Oncol*. 2009;27:3465-3471.
66. Bierman HR, Byron RL Jr, Kelley KH, Grady A. Studies on the blood supply of tumors in man. III. Vascular patterns of the liver by hepatic arteriography in vivo. *J Natl Cancer Inst*. 1951;12:107-131.
67. Visioni A, Kim M, Wilfong C, et al. Intra-arterial versus intravenous adoptive cell therapy in a mouse tumor model. *J Immunother.* 2018;41:313-318.
68. Katz SC, Burga RA, McCormack E, et al. Phase I hepatic immunotherapy for metastases study of intra-arterial chimeric antigen receptor—modified T-cell therapy for CEA+ liver metastases. *Clin Cancer Res.* 2015;21:3149-3159.
69. Katz SC, Hardaway J, Prince E, et al. HITM-SIR: phase Ib trial of intra-arterial chimeric antigen receptor T-cell therapy and selective internal radiation therapy for CEA+ liver metastases. *Cancer Gene Ther.* 2020;27:341-355.
70. Choi BD, Yu X, Castano AP, et al. CRISPR-Cas9 disruption of PD-1 enhances activity of universal EGFRvIII CAR T cells in a preclinical model of human glioblastoma. *J Immunother Cancer.* 2019;7:304.
71. Lin Y, Chen S, Zhong S, An H, Yin H, McGowan E. Phase I clinical trial of PD-1 knockout anti-MUC1 CAR-T cells in the treatment of patients with non-small cell lung cancer. *Ann Oncol*. 2019;30:xi12.
72. Wang Z, Li N, Feng K, et al. Phase I study of CAR-T cells with PD-1 and TCR disruption in mesothelin-positive solid tumors. *Cell Mol Immunol*. 2021;18:2188-2198.
73. Chen S, Lin Y. Phase I clinical trial using a unique immunotherapeutic combination of MUC1-targeted CAR-T cells with PD-1-knockout in the treatment of patients with advanced esophageal cancer. *J ClinOncol*. 2023;41:e16061.
74. Jaspers JE, Khan JF, Godfrey WD, et al. IL-18-secreting CAR T cells targeting DLL3 are highly effective in small cell lung cancer models. *J Clin Oncol*. 2023;133:e166028.
75. Hu B, Ren J, Luo Y, et al. Augmentation of antitumor immunity by human and mouse CAR T cells secreting IL-18. *Cell Rep*. 2017;20:3025-3033.
76. The ASCO Post Staff. Report outlines advance in retreatment with CAR T-cell therapy in patients with relapsed or refractory non-Hodgkin lymphoma or CLL. The ASCO Post. 2022. Available at https://ascopost.com/news/december-2022/report-outlines-advance-in-retreatment-with-car-t-cell-therapy-in-patients-with-relapsed-or-refractory-non-hodgkin-lymphoma-or-cll/. Accessed July 3, 2023.
77. Cirella A, Luri-Rey C, Di Trani CA, et al. Novel strategies exploiting interleukin-12 in cancer immunotherapy. *Pharmacol Ther.* 2022;239:108189.
78. Liao JB, Stanton SE, Chakiath M, et al. Phase 1/1b study of PRGN-3005 autologous UltraCAR-T cells manufactured overnight for infusion next day to advanced stage platinum resistant ovarian cancer patients. *J Clin Oncol.* 2023;41:5590.
79. Tselikas L, Champiat S, Sheth RA, et al. Interventional radiology for local immunotherapy in oncology. *Clin Cancer Res*. 2021;27:2698-2705.