Regional Gene Therapy for Cancer

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Introduction to Cancer Gene Therapy

Gene therapy is a powerful technology that holds significant promise for cancer treatment. Many strategies have been explored for gene therapy, including correction of mutant genes, immune stimulation, prodrug activation, interference of oncogene expression, cellular therapy, and the use of oncolytic viruses. One of the main obstacles limiting these therapies has been inefficient gene transfer, with subsequently poor expression. Achieving potent gene expression with the use of viral technology has been integral to the successes recently documented for chimeric antigen receptor (CAR) T-cell and oncolytic virus therapy.

The following sections will address each therapy in detail, beginning with CAR T cells and moving on to oncolytic viruses. We will highlight the principles and controversies related to these therapies, paying special attention to how each therapy is uniquely capable of optimizing key advantages of a regional delivery approach: *enhanced delivery of therapeutics to the site of the tumor*; *enhanced targeting of cancer cells, thereby limiting normal-tissue toxicity*; *and generation of both a local and a systemic immune response that can target metastatic disease and potentially prevent tumor recurrence*. Findings from investigational basic science literature will demonstrate the robust potential of these cancer-targeted gene therapies. For each therapeutic strategy, we will discuss the clinical trials that have used a regional delivery approach.

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CAR T-Cell Therapy

Principles and Application to Solid Tumors

Genetic engineering technology can be used to redirect T cells toward cancer antigens. The T cell is an ideal host cell: it divides rapidly, facilitating viral integration, has transcriptional machinery that promotes high-level transgene expression from viral promoters, and it can establish memory for long-lasting transgene expression. And it is also a robust antitumor effector cell: the signaling elements activated upon tumor antigen recognition trigger tumor lysis, as well as T-cell proliferation and cytokine secretion.

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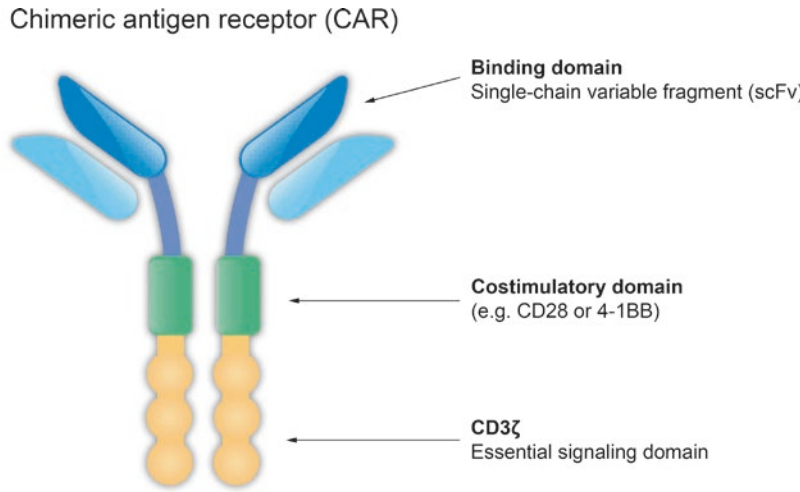

Fig. 5.1 Chimeric antigen receptors link an antibody-derived antigen-recognition domain to intracellular signaling domains that serve to activate and optimize T-cell function

CAR T cells are tumor-specific T cells generated by the transfer of genes encoding cancer-targeting receptors (Fig. 5.1) [1–4]. Retroviruses encode for these CARs and other cellular enhancements, serving as the delivery system for genome integration and subsequent expression. The "chimeric" namesake refers to the fusion of two separate protein domains; CARs link a high-avidity tumor antigen–binding element derived from a monoclonal antibody (which provides cancer cell recognition) to the CD3ζ intracellular signaling domain (to signal T-cell activation). This tandem fusion results in a high-avidity effective binding which then leads to phosphorylation of the intracellular signaling portion of the receptor, leading to T-cell activation [5–10]. Further genetic modification is then superimposed to optimize function. To provide both signals necessary to optimize T-cell proliferation and survival, signaling elements include costimulatory domains, such as CD28 and 4–1BB. Multiple studies have established that providing costimulation genetically encoded within the CAR is critical for the antitumoral activity of adoptively transferred T cells—enhancing both T-cell persistence and function [5–10]. The advent of so-called second-generation CARs, which combine activating and costimulatory signaling domains, has led to the successful use and subsequent FDA approval of two CD19-targeted CAR T-cell immunotherapies [11–13].

However, treating *solid tumors* requires overcoming multiple obstacles—achieving effective T-cell infiltration of a solid tumor mass that is highly immunosuppressive requires genetic modifications and delivery strategies that go beyond those of the original CAR design. The treatment of solid tumors is the focus of this chapter. We will highlight how regional delivery, which has the potential to efficiently deliver T cells to the primary tumor site and overcome tumor-mediated immune inhibition, is poised to become the optimal approach for CAR T-cell therapy. Furthermore, as with oncolytic virus therapy, the therapeutic benefit of regional delivery of CAR T cells extends beyond the local site of delivery. The generation of a local immune response can result in systemic immunosurveillance, with the potential to eliminate metastasis and prevent tumor recurrence.

Optimizing CAR T-Cell Therapy Using Regional Delivery

Solid malignancies pose unique obstacles to T-cell therapy. Unlike hematologic malignancies, which reside within the same peripheral compartment into which intravenously administered cells are delivered, solid tumor masses are sequestered within an immunosuppressive compartment that can be difficult to penetrate. Regional therapy can overcome the limitations

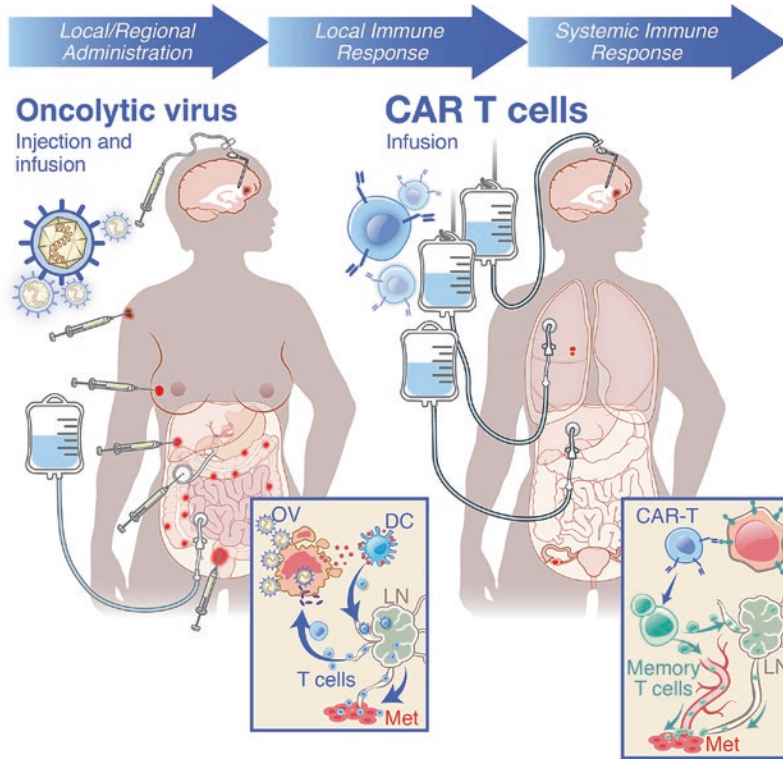

Fig. 5.2 [Left] Regional delivery of oncolytic virus (OV) is performed as either infusion or intralesional injection. Inset demonstrates viral infection of tumor cells leading to the two mechanisms of action of oncolytic viral therapy: (1) cancer cell death and (2) generation of a local and systemic antitumor immune response. Tumor cell death leads to release of damage-associated and pathogen-associated molecular patterns (DAMPs and PAMPs), triggering dendritic cell (DC) activation and migration to lymph nodes (LN), the anatomic location where T-cell priming occurs. Dendritic cells activate T cells by presenting tumor antigen and expressing activating cytokines and costimulatory ligands, leading to T-cell activation and differentiation. Effector T cells can now circulate to primary tumor, as well as to metastatic sites (MET), and effect immune-mediated tumor cell death. [Right] Regional therapy with chimeric antigen receptor (CAR) T cells employs either indwelling catheters or transient access to the delivery site to infuse cancer antigen targeted T-cell therapy. CAR T cells recognize cancer antigen, are triggered to activate, and induce regression of primary tumor. Activated T cells also generate T-cell memory that establishes a systemic immunosurveillance capable of inducing regression of metastases and preventing tumor recurrence

of systemic administration, enhancing tumor infiltration and overcoming immune suppression (Fig. 5.2). We recently demonstrated the merits of regional administration of mesothelin-specific CAR T cells in a clinically relevant model of pleural mesothelioma. Regionally administered—as compared with systemically delivered—CAR T cells displayed rapid and robust T-cell expansion and activation, with elimination of primary tumor [14]. Regional administration established circulation of CAR T cells that retained their functional activity, establishing T-cell memory and long-term systemic immunosurveillance capable of eradicating disseminated tumor sites. A single dose of regional CAR T-cell therapy provided effective protection against tumor rechallenge up to 200 days after the initial T-cell dosing; such persistence has been correlated with treatment efficacy and prevention of tumor relapse in several preclinical models and clinical trials. Based on these results, intrapleural administration of CAR T cells has now been translated to a phase I clinical trial of pleural mesothelioma and breast and lung primary tumors metastatic to the pleura (NCT02414269 and NCT02792114).

These results demonstrate that regional administration has benefits beyond treatment of the primary tumor. The ability of intrapleurally administered T cells to circulate and persist within the periphery opens new avenues of treatment for other metastatic cancers with accessible tumor sites, which may serve as a "regional charging and distribution centers" for CAR T-cell therapy—in effect, treating the most accessible tumor site can translate into sustained responses in more-inaccessible tumors. Examples of cancers that could benefit from this treatment strategy include those that metastasize to the pleural cavity (such as lung and breast cancers), those that metastasize to the peritoneal cavity (colorectal and ovarian cancers), and liver metastases (colorectal, gastric, and pancreatic).

Preclinical Data Supporting Regional CAR T-Delivery

Promising results using these approaches have been seen in preclinical studies of intracranial, intraperitoneal, and intrahepatic delivery of CAR T-cell therapy.

Our group at Memorial Sloan Kettering has shown that intrapleurally administered CAR T cells show enhanced antitumor efficacy in an orthotopic mesothelioma mouse model even at a reduced dose compared to systemically administered CAR T cells, and this enhanced efficacy is facilitated by CD4-dependent CD8 T-cell proliferation [15]. A group at Roger Williams Medical Center demonstrated that intraperitoneal administration of carcinoembryonic antigen (CEA)–targeting CARs, in an animal model of colorectal primary tumor with peritoneal carcinomatosis, was superior to intravenous administration and was able to mediate regression of extraperitoneal tumor sites [16]. Other groups have similarly shown robust antitumor activity following intraperitoneal administration of CAR T cells [17]. A group from Sloan Kettering Institute showed that intraperitoneal administration of IL-12–secreting CAR T cells was efficacious in a model of MUC16-expressing ovarian peritoneal carcinomatosis. IL-12 secretion was genetically encoded in the same viral vector used for CAR transduction, simplifying gene delivery and optimizing CD8+ T-cell function [18]. Hepatic vascular infusion is another promising avenue of regional administration, as demonstrated by the group at Roger Williams. The researchers infused CAR T cells into the portal circulation in a model of CEA-expressing colorectal liver metastases [19].

Despite these preclinical successes, obstacles remain in the treatment of solid tumors. The absence of T cells 2 weeks after initial administration in a clinical trial targeting glioblastoma suggested that, even with the robust T-cell infiltration achieved by regional delivery, CAR T cells may not be able to overcome all of the challenges that solid tumors present [20]. An increasing amount of preclinical and clinical experience has demonstrated the importance of overcoming tumor-mediated immune inhibition, which is the focus of the next section.

Gene Engineering to Enhance Efficacy: Engineering the T Cell Beyond the Car, with a Focus on Overcoming Immune Suppression

The immunosuppressive obstacles encountered by CAR T cells led to the realization that large, established solid tumors require CAR T cells with enhancements that go beyond CAR recognition and signaling. Although regionally delivered T cells may infiltrate the tumor mass more efficiently than systemically delivered T cells, they are still subdued by a formidable immunosuppressive tumor environment upon arrival. Various groups have taken advantage of the flexibility afforded by viral vectors to further enhance CAR T-cell function, creating T cells that optimize T-cell metabolism [6, 9], program a stem cell–like pattern of expression that enhances survival and self-renewal [20, 21], and express cytokines and cytokine receptors that optimize function [1, 22].

One of the more compelling strategies is engineering T cells to overcome tumor-mediated immune inhibition. To eliminate tumor cells, T cells must not only persist but must sustain function in an environment rich with inhibitory

signaling. The success of antibodies targeting immune checkpoints such as programmed death 1 (PD-1) and cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) supports the therapeutic potential of counteracting immune inhibition [23–25]. Since adoptively transferred T cells are susceptible to inhibition, strategies combining adoptive T-cell therapy with checkpoint blockade have been investigated [26–28]. In "adaptive immune resistance" [29], tumor cells generate anti-immune protection by expressing coinhibitory ligands, such as PD-1 ligand, following exposure to T-cell–secreted Th1 cytokines [30–32].

Our own preclinical data established that human CAR T cells—even when combined with costimulatory signaling with either 4–1BB or CD28—are subject to inhibition. Using a model of pleural mesothelioma, we demonstrated that T-cell exhaustion can be reversed by interfering with the PD-1 pathway, either by antibody blockade or by genetically engineering CAR T cells to overexpress a PD-1 dominant negative receptor (which serves as a decoy receptor to prevent signaling through the native PD-1 receptor) or an shRNA-targeting PD-1 receptor (which downregulates PD-1). Whereas both avenues of checkpoint blockade are effective, the genetically engineered strategy might be preferred for its efficacy and simplicity, as it nullifies the need for repeated antibody administration. Other groups have also developed strategies to overcome CAR T-cell inhibition in solid tumors [32, 33]; examples include the use of IL-12–secreting CAR T cells to overcome PD-1–mediated inhibition [34], a PD1CD28 "switch receptor" that translates inhibitory ligand binding into costimulatory signaling [35], CRISPR/Cas9 gene editing to generate PD-1–deficient CAR T cells [36], and the construction of T cells that secrete PD-1 antibody [37].

Safety Engineering for CAR T-Cell Therapy

Retroviruses have been primarily used in CAR T-cell therapy to deliver the genetic sequence encoding the CAR. The payload is delivered as RNA that is then reverse-transcribed into DNA for permanent integration into the genome of patient cells. Although integration provides high fidelity and long-lasting expression, it also carries the potential for insertional mutagenesis and malignant transformation. Lentiviral vectors have a safer integration site profile than gammaretroviral vectors [38]; however, both have been safely used at major US institutions that are pioneering CAR technology [39, 40].

Although CD19 CAR T cells have shown impressive efficacy in treating leukemia, this success has come with significant and at times life-threatening side effects due to T-cell cytokine release leading to a systemic inflammatory response that manifests as fever, hypotension, and neurologic dysfunction. Most cases of *cytokine release syndrome* can be treated with corticosteroids and IL-6–targeting antibodies, with the occasional need for end-organ support in the intensive care unit. For increased safety, "suicide genes" such as iCaspase-9 [41], EGFR (epidermal growth factor receptor) mutation [42], and herpes simplex virus (HSV) thymidine kinase [43] can be used to mediate rapid T-cell elimination after administration of a prodrug or antibody, should side effects persist.

Most CARs targeting solid tumors are aimed at antigens shared by normal tissues and, therefore, carry the risk of "on-target off-tumor toxicity" [44, 45]. Judicious selection of the target antigen can offset this. The optimal target is one whose expression is restricted to expendable cells or, better yet, to tumor cells only. Examples of suitable targets include mesothelin (we have not experienced any on-target off-tumor toxicities in our ongoing phase I clinical trial), the mutated form of EGFR that is expressed on glioblastoma multiforme tumors [46, 47], and a glycosylated form of MUC1 that is unique to tumor cells [48, 49]. Genetic strategies to limit normal-tissue toxicity are also active areas of investigation [50, 51].

Clinical Trials of T Cells Using Regional Delivery Strategies

Early phase clinical trials employing regional delivery have now emerged. Hepatic arterial

infusion, achieved through percutaneous access of the arterial system using angiographic catheters, has been investigated as a method of delivery of CEA-targeting CAR T cells for the treatment of colorectal liver metastases. Hepatic arterial infusion was well-tolerated (NCT01373047) [52]; intravenous delivery, on the other hand, was associated with dose-limiting colitis (NCT00923806) [53]. All but 1 patient in the HAI study had more abundant CAR T cells in liver metastasis tissue compared to healthy liver tissue, including 1 patient who had a durable presence of CAR T cells 12 weeks after initial administration. Furthermore, CAR T cells were detected in peripheral blood samples from only 2 of 8 patients. Although hepatic arterial infusion may result in decreased toxicity, the limited systemic immunity generated may ultimately limit efficacy, especially for extrahepatic disease. As we and others have demonstrated in preclinical studies, systemic T-cell immunity focused on a safer target than CEA may be the more optimal approach, achieving both efficacy and safety [15]. The authors of the HAI study assessed response to treatment by monitoring CEA levels, as imaging studies often do not adequately reflect response to immunotherapy. Although seven of eight patients in this trial had some decrease in CEA level, all but one had died at the time of study publication, with a median overall survival of 15 weeks.

In a trial from City of Hope (NCT00730613) [54], IL13Rα[alpha]2-targeting CARs were infused directly into 3 patients with brain tumors, using a catheter/reservoir system. Treatment was well-tolerated and displayed some antitumor activity: 1 patient had decreased target antigen expression, and another had an increase in necrosis as measured by MRI. A second publication from this group [55] describes a patient with multifocal glioblastoma recurrence including multiple brain and spinal metastases who was initially treated with CAR T cells infused directly at a resected brain tumor site via catheter infusion. Although there was no relapse at the infused resection cavity, other lesions progressed. A remarkable response came when T cells were infused into the cerebrospinal fluid by accessing the lateral ventricle, a delivery method associated with complete radiographic elimination of spinal metastases and a good response in brain metastases. This response was durable to 7.5 months, and measurable T cells were present along with cytokines in the cerebrospinal fluid for at least 7 days after each ventricular infusion.

An ongoing trial from our group at the Memorial Sloan Kettering has demonstrated safety and promising antitumor activity of intrapleurally administered CAR T cells targeting mesothelin expressed on cancer cells. Although we have clearly seen indications of the potential for efficacy of regional delivery of CAR T cells, clinical trials exploring this approach have thus far been conducted with limited numbers of patients (Table 5.1). We therefore await more-mature results to further clarify the efficacy of regional delivery of CAR T-cell therapy.

Oncolytic Virus Therapy

Background, Viral Technologies, and Regional Delivery to Solid Tumors

Oncolytic viruses are versatile, capable of direct lysis of the tumor, and able to deliver transgenes to enhance efficacy and decrease toxicity. As one of the mechanisms of oncolytic efficacy is tumor cell lysis, replication-competent viruses are specifically chosen for their ability to self-replicate and reinfect. The experience of using oncolytic viruses to treat cancer has shown they not only induce tumor cell lysis but also generate antitumor immunity, both of which contribute to treatment effect. Furthermore, as with CAR T cells, oncolytic viruses can be genetically engineered to express therapeutic transgenes that further enhance antitumor activity.

The benefits of regional delivery of oncolytic viruses are similar to those for CAR T cells (Fig. 5.2). Researchers at Massachusetts General Hospital demonstrated that intraperitoneal administration of an oncolytic HSV for peritoneal metastases (colorectal primary) achieved better tumor lysis than systemic delivery. An added benefit of regional delivery was decreased

Table 5.1 Completed CAR T trials with regional delivery strategy

	NCT Year launched Phase Center Number of patients	CAR target Costimulation Other features	Cancer diagnosis	Administration	Notable study feature or finding
1	NCT00730613 [54] 2008 Phase 1 City of Hope medical center 3 patients	IL13Rα[alpha]2 NKG2D+/-CD28 costimulation Hy/TK selection/suicide fusion protein	Glioblastoma	Tumor resection cavity via catheter/ reservoir system	All patients had increased FLAIR MRI inflammatory signal after treatment, and 1 patient with re-resection had decreased tumor IL13Rα[alpha]2 expression. Both observations suggest an efficacy signal which correlated with level of IL13Rα[alpha]2 antigen expression. Due to 3 grade 3 AEs, dose escalation terminated. No ≥ grade 4 AEs
2	NCT01082926 [95] 2010 Phase 1 City of Hope medical center 6 patients	IL13Rα[alpha]2 CD28 costimulation Hy/TK selection/suicide fusion protein	Glioblastoma	Intratumoral infusion	CAR T cells were detected at the site of recurrence near corpus callosum in one patient, suggesting that CAR T cells can traffic to other sites of disease
3	NCT01373047 [52] 2011 Phase 1 Roger Williams medical center 8 patients	CEA CD28 costimulation	Colorectal and Periampullary adenocarcinoma with liver metastases	Hepatic arterial infusion using percutaneous access	CEA levels were used to monitor efficacy to overcome limitations of imaging studies in assessing response to immunotherapy No ≥ grade 3 AEs related to the CAR T-hepatic arterial infusions
4	NCT01837602 [96] 2013 Phase 0 University of Pennsylvania 6 patients	c-met 4–1BB costimulation	Breast	Intratumoral injection	Extensive necrosis at injection site and loss of c-met expression both suggest efficacy 6 ≥ grade 3 AEs
5	NCT02208362 [55] 2015 Phase 1 City of Hope medical center Ongoing patient recruitment	IL13Rα[alpha]2 4–1BB costimulation	Glioblastoma	Intratumoral, intracavitary, intraventricular	Ongoing study with case report of patient who received dual intracavitary and intraventricular therapy and had transient complete response for 7.5 months No ≥ grade 3 AEs

toxicity to normal tissues [56], enabling higher doses. Other examples of regional delivery of oncolytic viruses in the preclinical setting include portal infusion of HSV in a model of colorectal liver metastases [57], carotid infusion of HSV to treat head and neck squamous cell carcinoma [58], intrapleural administration of HSV to treat pleural-based lung cancer [59, 60], and intraperitoneal administration of a vaccinia virus to treat malignant peritoneal mesothelioma [61].

Oncolytic Viruses Generate Both a Local and a Systemic Immune Response

Although classified as a local intervention, treatment with oncolytic virus can elicit a systemic antitumor immune response, serving as an in vivo vaccine that generates a local innate and adaptive immune response with the potential of establishing systemic immunity.

The progression from a local immune response to systemic immune surveillance follows the typical immunologic sequence of triggering innate immunity followed by activation of adaptive immunity. Oncolytic viruses induce highly immunogenic cell death whereby tumor cell lysis leads to local efflux of tumor antigens and danger signals that trigger antigen-specific immunity: dendritic cells are recruited for antigen uptake (and activated by cell breakdown products) and migrate to lymph nodes to activate the adaptive immune system. The end effector is the potent T cell, which mediates antitumor effect via tumor lysis and cytokine secretion. The response induced by T cells can be particularly robust if tumor antigens that are especially immunogenic are made available; the availability of these antigens (termed "neoantigens") depends on the mutation frequency found within the tumor. The importance of these neoantigens has been highlighted by studies demonstrating that unique mutations identified by tumor sequencing identify patients likely to respond to immunotherapy [62, 63]. In effect, oncolytic viruses are in vivo vaccinations that generate systemic immunity capable of inducing regression of distant, uninjected/uninfected tumors [64]. In other preclinical studies, mice previously cleared of tumor by the use of an oncolytic virus remained tumor-free after rechallenge with tumor cells, which is consistent with the establishment of immunologic memory and suggests that oncolytic viruses can play a role in preventing recurrence [65, 66]. Such findings are consistent with our observations that regional delivery of CAR T-cell therapy generates a systemic response by establishing circulating T-cell memory.

The ability of oncolytic viruses to generate a de novo endogenous immune response supports the use of rational combinations of oncolytic viruses and immune checkpoint blockade (ICB) agents. As the efficacy of ICB depends on the activation of a preexisting immune response [23, 67–69], delivery of oncolytic virus can be used to turn a "cold" tumor into a "hot" tumor with a pro-inflammatory/immunogenic environment, which can be followed by ICB to release the "brakes" on the antitumor T-cell immune response [70, 71]. Preclinical studies have demonstrated that intralesional injection of oncolytic virus can induce T-cell infiltration and increase the efficacy of CTLA-4 blockade in melanoma tumors; the combination of intralesionally administered oncolytic virus and CTLA-4 blockade enhanced regression of both injected and distant metastases [64, 72].

Safety Engineering for Oncolytic Viruses: Enhancing Tumor Tropism for Selective Replication in Tumor Cells

As we have demonstrated for CAR T cells, the clinical promise of oncolytic virus therapeutics relies on safety as much as efficacy. Safety is of particular concern in the case of oncolytic viruses, as these viruses are infectious pathogens that can cause disease. By regulating the viral life cycle of oncolytic viruses—manipulating attachment, cell cycle entry, and viral replication—they can be optimized to selectively target tumor cells.

Genetic modification can alter the viral capsid to enhance binding to cell-entry receptors preferentially expressed on tumor cells [73] and even exchange the typical capsid epitopes for single-

chain variable fragments that target virus to a tumor cell surface receptor of choice [74]. Another way to preferentially lyse tumor cells is to preferentially spare normal tissue; the deletion of virulence genes such as thymidine kinase can result in selective replication within only rapidly dividing tumor cells that have sufficient transcriptional machinery to support viral replication [75]. The only FDA-approved oncolytic virus therapy—T-VEC—is a modified HSV-1 with ICP34.5 inactivation, which halts replication and leads to apoptosis of infected normal cells [65, 76]. Another deletion strategy is to place virulence genes under the control of tumor tissue–specific promoters [77–79].

Genetic Engineering to Express Therapeutic Genes

Oncolytic viruses are extremely versatile therapeutics, as they can be genetically engineered with elements that enhance their two primary mechanisms of action: lytic function and stimulation of antitumor immunity. Serving as a vector for the delivery of therapeutic genes, T-VEC secretes granulocyte macrophage colony-stimulating factor (GM-CSF), which recruits and activates dendritic cells for optimal antigen presentation to T cells. Other strategies aimed at bolstering the immune response include delivery of cytokines (IL-2, IL-12, TNF) [80, 81], expression of tumor-associated antigens [74], and the addition of costimulatory signaling [72, 82]. To enhance tumor lysis, transgenes have been incorporated (1) to activate chemotherapy prodrugs [83–85], and (2) to express thymidine kinase (which converts administered ganciclovir into the toxic ganciclovir monophosphate) [86].

Clinical Trials of Oncolytic Viruses Using Regional Delivery Strategies

To date, only one oncolytic virus—T-VEC, an attenuated HSV-1 engineered to express GM-CSF—has been approved by the FDA for the treatment of cancer, specifically for advanced melanoma. Promising early clinical results led to the first randomized controlled trial: OPTIM (Oncovex$^{GM\text{-}CSF}$ Pivotal Trial in Melanoma) [87–89]. In patients with stage IIIb, IIIc, or IV melanoma with unresectable but accessible lesions, treatment with T-VEC resulted in an enhanced durable objective response (16.3% vs. 2.1%; $p < 0.001$) and overall response (26.4% vs. 5.7%; $p < 0.001$) rate, compared with recombinant GM-CSF. Regression was observed in injected and uninjected lesions, which supports the role of oncolytic viruses to generate systemic immunity [90]. Following these results, the FDA-approved T-VEC for the treatment of unresectable, injectable cutaneous, subcutaneous, and nodal melanoma with limited visceral disease. Locally delivered intralesional T-VEC generated an antitumor immune response; injected lesions accumulated MART-1–specific $CD8^+$ T cells, with an associated decrease in $CD4^+FoxP3^+$ regulatory T cells and myeloid-derived suppressor cells [90, 91].

Many of the preclinical studies supporting combination therapy with an oncolytic virus and an ICB agent have now been translated into clinical trials (Table 5.2). The use of intralesional T-VEC followed by the anti–CTLA-4 antibody ipilimumab in patients with advanced melanoma [92] resulted in an objective response rate of 50%, with 44% of the patients exhibiting durable responses lasting >6 months. A subsequent randomized controlled trial (comprising 198 patients with unresectable stage IIIB-IV melanoma) compared T-VEC with and without ipilimumab and found a significant difference in the response rate (39% vs. 18%; $p = 0.002$); correlative studies found increased levels of T cells in patients receiving T-VEC with ipilimumab [93]. The use of the anti–PD-1 antibody pembrolizumab may be even more effective—in a phase I study of 21 patients with melanoma, pembrolizumab resulted in a 62% objective response rate and an impressive 33% complete response rate (NCT02263508) [94].

Preclinical and clinical studies have explored other methods of delivery for oncolytic virus therapy, including pleural and peritoneal delivery and hepatic arterial infusion. These methods have been combined with systemic therapy, including chemotherapy and ICB. Intraperitoneal delivery

Table 5.2 Selected oncolytic trials with Regional Delivery Strategy with published results and novel study findings or features

	NCT Year launched Phase Center Number of patients	Virus design	Cancer diagnosis	Notable study feature or finding
Intrahepatic				
1	NCT00012155 [97] 2003 Phase 1 MSK 12 patients	NV 1020	Colorectal	A majority of virus cleared by the liver and not found in systemic circulation, proving advantage of regional delivery 1 SAE attributed to viral therapy
Intraperitoneal				
1	NCT00002960 [98, 99] 1997 Phase 1 Multicenter 36 patients	Recombinant adenovirus-p53 SCH-58500	Fallopian tube, ovarian, primary peritoneal	50% of women who completed 3 cycles of treatment had a CA-125 response, used to monitor responses 8 ≥ grade 3 AEs
2	NCT00408590 [100] 2004 Phase 1 Mayo Clinic, NCI 37 patients	CEA-expressing measles virus with thyroidal sodium iodide symporter	Ovarian	Dose-dependent CEA elevation was observed in peritoneal fluid and serum, supporting dose- dependent activity 5 patients had significant decreases in CA-125 levels, used to monitor responses 3 SAEs in cohort 1, 2 SAEs in cohort 2
Intrapleural				
1	NCT01212367 [101] 2009 Phase 1 UPenn, NCI 9 patients	Ad.hIFN-α[alpha] (Scheme 721015, adenoviral-mediated interferon alpha)	Mesothelioma	No increase in humoral immune response to the virus antigen or mesothelin, however there was response to the mesothelioma cells. Two patients subsequently underwent radical pleurectomy Dose escalation terminated due to severe "flu-like" symptoms. Two patients had catheter infections
2	NCT01119664 [102] 2010 Phase 1,2 UPenn 40 patients	Ad.hIFN-α[alpha] (Scheme 721015, adenoviral-mediated interferon alpha)	Mesothelioma	Median overall survival for all patients with epithelial histology was 21 months versus 7 months for patients with non-epithelial histology. For both cohorts combined, there was stable disease in 62.5% of patients and partial responses in 25% of patients, however, no complete responses were observed 6 SAEs, none attributable to drug instillation
Intratumoral				
1	NCT00289016 [103] 2005 Phase 2 Multicenter 50 patients	HSV with GM-CSF (Talimogene laherparepvec)	Melanoma	26% of patients on study got to NED (3 able to have surgery after T-VEC). 1-year survival was achieved for all patients who had partial response, complete response, or surgical complete response No SAEs related to treatment

Table 5.2 (continued)

	NCT Year launched Phase Center Number of patients	Virus design	Cancer diagnosis	Notable study feature or finding
2	NCT00769704 [104] 2009 Phase 3 Multicenter 436 patients	HSV with GM-CSF (Talimogene laherparepvec)	Melanoma	26% of patients treated with T-VEC had OR, vs. 6% of patients treated with GM-CSF No ≥ grade 3 AE occurred in ≥3% of pts. in either arm
3	NCT01740297 [88] 2013 Phase 1,2 Multicenter 217 patients	HSV with GM-CSF (Talimogene laherparepvec)	Melanoma	The ORR of the combination therapy group was 38.8% vs. 18% with ipilimumab alone. 13.3% of patients in the combination group achieved complete response (vs. 7%) Combination therapy group had a higher rate of response in uninjected lesions (35.5% vs. 13.6%) 28% of combination therapy patients and 18% of ipilimumab patients had ≥ grade 3 AE
4	NCT02263508 [105] 2014 Phase 1b Multicenter 21 patients	HSV with GM-CSF (Talimogene laherparepvec)	Melanoma	Circulating CD8+ T cells, including those expressing Tim3 and BTLA became elevated during treatment with T-VEC initially but decreased after pembrolizumab began 33% of patients had grade 3 or 4 AEs
5	NCT00554372 [106] 2008 Phase 2 Multicenter 30 patients	JX-594: Recombinant vaccinia virus (TK-deletion plus GM-CSF)	Hepatocellular	Assessed induction of humoral antitumor immunity through antibody—Mediated complement dependent toxicity (CDC). 11/16 patients in the high-dose cohort developed CDC. Also assessed cellular immunity and found that cytotoxic T cells were induced to vaccinia peptides and the JX-594 transgene product β-gal 4/14 patients in low dose and 4/16 patients in high dose had SAEs
6	NCT01227551 [107] 2011 Phase 2 Multicenter 57 patients	Coxsackievirus A21 (CVA21)	Melanoma	Both injected and uninjected lesions responded. 14/40 evaluable patients (35%) achieved irPFS at 6 months No ≥ grade 3 or 4 product related AEs
7	NCT02272855 [108] 2014 Phase 2 Multicenter 46 patients	HF10	Melanoma	Responding tumors showed increased total TILs and CD8+ T cells 3 ≥ grade 3 AEs

AE, adverse event; *BTLA,* B and T lymphocyte associated; *CEA*, carcinoembryonic antigen; *GM-CSF*, granulocyte macrophage–colony stimulating factor; *HSV*, herpes simplex virus; *NED*, no evidence of disease; *SAE*, severe adverse event; *T-VEC*, talimogene laherparepvec; *TK*, thymidine kinase

can be used for primary tumors presenting as carcinomatosis. Early studies have demonstrated the safety and feasibility of intraperitoneal delivery. Patients with ovarian or primary peritoneal cavity cancers have received intraperitoneal delivery of an adenovirus (NCT00002960) via Hickman, Tenckhoff, or PortaCath catheters. The authors observed manageable toxicity and found transgene expression in both ascitic fluid and tumor biopsy specimens. Similar to the difficulties faced when attempting to estimate the peritoneal surface disease during preoperative evaluation, CT scan did not reliably identify an effect on disease, and measured CA-125 levels may be a better way to gauge treatment response in this context. Combination treatment with chemotherapy performed the best, and a dose-dependent effect was observed. Regional therapy has also shown promise for malignant pleural mesothelioma. Intrapleural delivery (via pleural catheter) of adenovirus with IFN-γ (NCT01119664) and adenovirus with IFN-α[alpha] (NCT01212367) have been safely applied in early phase clinical trials. One patient from the latter study had a significant response at both intra- and extrathoracic disease sites, suggesting that systemic immunity was generated. Hepatic arterial infusion is a potential regional delivery method for primary and metastatic lesions to the liver. In NCT00012155, patients received an intraarterial injection of NV1020 (HSV) into the hepatic artery for the treatment of colorectal liver metastases.

Conclusion

As our understanding of solid tumor immunology deepens, and as genetic engineering technology continues to advance, regional gene therapies are poised to become effective options in cancer treatment. Regional gene therapy may be an essential first step to achieving durable responses in solid tumors. The ability to generate both local and systemic antitumor immunity is an especially promising attribute of these treatments. Solutions to avoiding normal-tissue toxicity have been addressed in preclinical studies and have already been translated to the clinic. Combination treatment strategies, incorporating chemotherapy, radiation therapy, and immunotherapies, will serve to enhance efficacy.

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