REVIEW ARTICLE

Combination therapy with CAR T cells and oncolytic viruses: a new era in cancer immunotherapy

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Chimeric antigen receptor (CAR) T-cell therapy is an encouraging and fast-growing platform used for the treatment of various types of tumors in human body. Despite the recent success of CAR T-cell therapy in hematologic malignancies, especially in B-cell lymphoma and acute lymphoblastic leukemia, the application of this treatment approach in solid tumors faced several obstacles resulted from the heterogeneous expression of antigens as well as the induction of immunosuppressive tumor microenvironment. Oncolytic virotherapy (OV) is a new cancer treatment modality by the use of competent or genetically engineered viruses to replicate in tumor cells selectively. OVs represent potential candidates to synergize the current setbacks of CAR T-cell application in solid tumors and then and overcome them. As well, the application of OVs gives researches the ability to engineer the virus with payloads in the way that it selectively deliver a specific therapeutic agents in tumor milieu to reinforce the cytotoxic activity of CAR T cells. Herein, we made a comprehensive review on the outcomes resulted from the combination of CAR T-cell immunotherapy and oncolytic virotherapy for the treatment of solid cancers. In the current study, we also provided brief details on some challenges that remained in this field and attempted to shed a little light on the future perspectives.

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INTRODUCTION

Besides the use of conventional treatment strategies, such as chemotherapeutic drugs, radiotherapy, and surgery, immunotherapy has also attracted many attentions recently to be used as an efficient strategy to treat cancer. Among different immune-modulatory approaches, checkpoint inhibitors and chimeric antigen receptor (CAR) T cells were found to eliminate the population of cancer cells through both reinforcing and recruiting the host's immune system potential against cancer cells [1]. While the first 50 years of immunotherapy's history experienced many successes and failures, the era since 2010 has seen a collection of evident achievements that resulting in the commercialization of several products [1], including the first approvals in the USA and European Union. Furthermore, immunotherapy due to its confirmed promising efficacy in cancer therapy, received Nobel Prize of Medicine in 2018. Adoptive immunotherapy with T lymphocytes, genetically modified to express chimeric antigen receptors (CARs), is known as a promising and fast-growing strategy used for the treatment of recurring and aggressive malignancies [2]. CARs are genetically engineered fusion molecules generated through combining the antigen recognition capacity of a specific antibody with the immune cells effector function, which cannot only detect the target antigen expressed on the surface of tumor cells, but can also proliferate and kill the targeted neoplastic cells [3, 4]. CAR T cells can identify antigenic epitopes in their unprocessed form, which have not been previously presented to T cells [5]. Accordingly, MHC-independent antigen recognition countervails immune evasion pathways through which cancer cells evade MHC-restricted T-cell detection, such as defects in antigen processing or the diminished expression of MHC molecules [6].

In recent years, CAR T cells have been widely used in different hematologic malignancies and due to their success in improving the outcome of patients, FDA approved them for the treatment of lymphoma and acute lymphoblastic leukemia (ALL) [7]. Unlike hematologic malignancies, the application of CAR T cells in solid tumors was found to be along with several challenges such as the frailty of CAR T cells under the immunosuppressive conditions of the tumor microenvironment (TME), limited trafficking ability of CAR T cells, heterogeneity and the difficulty in finding the ideal target tumor antigens, antigen-negative relapse, and the decreased persistence and expansion of CAR T cells [8, 9]. The poor outcomes of CAR T cells in preclinical and clinical trials of solid tumor indicated that monotherapy with CAR T cells is not enough for the treatment of such malignancies and the combining them with other adjuvant therapies might bring more advantageous for patients with cancer [10].

An idea of employing genetically engineered viruses with the selective replicative preference in tumor cells has evolved the introduction of a novel cancer treatment approach, named as oncolytic virotherapy [11]. The golden age of oncolytic virotherapy has begun when Talimogene laherparepvec (T-VEC), which is an oncolytic herpes simplex virus type 1 (HSV-1) engineered to

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express GM-CSF [12], received the FDA approval for the treatment of patients with advanced melanoma in combination with immune checkpoint inhibitors [13]. In particular, it was shown that oncolytic viruses (OV) exert their anticancer activities either through directly affecting tumor cells or through mobilizing the tumor surveillance immune responses against the neoplastic cells [14, 15]. Another advantage caused by OVs for cancer treatment strategies is that these viruses could be engineered in a way to force the expression of some genes in tumor milieu, which eventually either potentiate their oncolytic property or enhance the antitumor arm of immune responses [16]. All the above-mentioned statements make OVs a promising candidate used to overcome the current setbacks of CAR T-cells application in solid tumors [15, 17]. This study aimed to focus on more recent advances in both preclinical and clinical applications of OVs, either alone or in combination with CAR T-cells approaches.

ONCOLYTIC VIROTHERAPY—AN OVERVIEW

After the revolutionizing impact of immune checkpoint inhibitors on cancer treatment strategies, it seems that oncolytic virotherapy could push the borders a step-forward. These novel treatment approaches well-performed in the treatment of various cancers (Table 1). Through selectively replicating in tumor cells as well as concurrent renovating antitumor immunity, application of OVs could guarantee that the normal non-malignant host cells would not be affected at least [18–20]. Talimogene laherparepvec (T-vec) was the first OVs-based agent gaining the approval of both USA and Europe. Accordingly, this product was engineered based on the oncolytic herpes simplex virus (HSV), which has that acquired the ability of producing granulocyte-macrophage colony-stimulating factor (GM-CSF), while lost the potential of expressing ribonucleotide reductase [21]. A randomized phase III study have previously demonstrated that local intralesional inoculations with T-Vec in patients with advanced melanoma were coupled not only with tumor repression, but also with the prolonged overall survival (OS) of patients, probably by inducing a systemic antitumor immune response [12]. Other oncolytic products with a great chance of being approved in North America and Europe, are as follows: GM-CSF armed adenovirus CG0070 for nonmuscle invasive bladder cancer [22], vaccinia virus JX-594 (pexastimogene devacirepvec) for colorectal cancer [23] and hepatocellular carcinoma [24], and Reolysin (pelareorep), which is a wild-type variant of reovirus, for metastatic melanoma [25] and Pancreatic Adenocarcinoma, respectively [26].

By inducing a deletion mutation in the genome of G207, which is known as the second-generation HSV-1, the third generation of oncolytic HSV-1, named as G47Δ, was produced [27]. The efficacy and therapeutic value of this oncolytic virus is currently under investigation in a phase II of a clinical trial conducting on patients with recurrent glioblastoma (UMIN000015995). However, G47Δ was appointed by the Ministry of Health, Labour, and Welfare of Japan in 2016 as a "Sakigake" breakthrough therapy drug [28]. HF-10, which is another HSV-1-based oncolytic agent, was found to lack the expression of various genes of the virus initially, including LAT (latency-associated transcripts), UL43, UL55, UL56, and UL49.5 [29]. Moreover, this oncolytic virus, engineered by Takara Bio Inc., Japan, selectively replicates in tumor cells and then stimulates an enhanced number of NK cells, CD4+, and CD8+ T cells within the tumor microenvironment, which consequently leads to a significant decrease in the tumor size [30]. Similar to T-VEC, HF-10 has also been observed to have a potent antitumor effect on a wide range of cancers [31] and several clinical trials with HF-10 are ongoing or have been designed, such as melanoma (NCT02272855), and unresectable pancreatic (NCT03252808) (Table 1). Furthermore, the vaccine strain of the measles virus has demonstrated several considerable outcomes in both previously performed preclinical and clinical trials [32, 33].

It is well established that tumor-specific viral replication and subsequently the induction of tumor-specific immunity are the two main reasons that guaranteeing the success of OVs in improving the median survival of cancer patients [34]. Regarding safety, therapeutic efficacy, and the decreased side effects of oncolytic virotherapy, the use of such modified viruses in biological therapy of cancer was observed to have the potential of assigning a breakthrough in the field of cancer research.

CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY IN SOLID TUMOR

Chimeric antigen receptors are artificial receptor proteins genetically engineered from the fusion of a single-chain variable fragment (scFv) in a monoclonal antibody (as an extracellular antigen recognition domain) along with the ζ -chain of the T-cell receptor complex (as an intracellular signaling domain) as well as one or two co-stimulatory molecules [35]. CARs are classically transduced into the patient T cells applying γ-retroviral vectors or other randomly incorporating vectors, which may lead to oncogenic transformation, clonal expansion, transcriptional silencing, and variegated transgene expression [36–39]. Recent progresses in genome editing technology facilitate efficient sequence-specific interventions in various cells [40]. Eyquem et al. demonstrated that integrating a CD19-specific CAR to the TRAC (T-cell receptor α constant) locus not only results in constant CAR expression in patients' blood T cells, but also increases T-cell potency [36].

The logic of CAR T immunotherapy mainly is changing T cells, in order to identify malignant cells for more efficiently recognizing and destroying them. Following CAR T cells infusion into a cancerous patient, they would act as a "living drug" against malignant cells [41]. Once CAR T cells come in contact with their targeted antigens on the surface of the tumor cell, they bind to it, become activated, and finally destroy target cells [42].

Although CAR T-cell therapy exhibited positive results in hematological malignancies, the application of this approach in solid tumors has faced several obstacles [43, 44]. Indeed, the results of the earlier studies that evaluated the potential of CAR T-cell immunotherapy in patients with solid tumor, revealed that this strategy has minimal to limited anticancer properties (Table 2) (Fig. 1). One of the examples of investigations that evaluated the efficacy of CAR T cells was a study conducted by Kershaw et al. [45] who performed a treatment for patients with metastatic ovarian cancer with the first generation of CAR T cells targeted alpha-folate receptor (FR) and high dose of IL-2. Their results suggested that although this combination therapy was well-tolerated by patients, CAR T cells could not actually induce significant therapeutic effects on patients and on preventing disease's progression, as their circulating level significantly decline within 3 weeks after infusion [45]. It could be claimed that the application of fist-generation CAR T-cell clone probably was the main reason of why this trial failed to achieve acceptable results due to lack of co-stimulation. However, even by the use of a high later generation of CAR T-cells products, objective response for trials in solid tumors have been mostly disappointing (Table 2). Apart from that, several other reasons could also suggest for why early CAR T-cell products could not perform well in solid tumors [46, 47]. Of note, unlike hematologic malignancies, solid tumors demonstrate unparalleled setbacks. In this regard, the first and the most important reason was found to be the absence of constantly expressed specific tumor antigens on the surface of solid tumors. Second, infused CAR T cells must pass a long way from the bloodstream to solid tumor regions, penetrate into the dense and tough stroma and also match with chemokine receptors. Third, upon the arrival of TME, the most of CAR T cells overwhelm the challenges and immunosuppressive factors in order to expand, infiltrate, and elicit tumor-specific cell cytotoxicity. As the fourth

Table 1. Summary of major OVs under clinical development in solid tumors.

Oncolytic agent	Virus type	Route of administration	Gene insertion	Clinical phase	cancer type	Study Sponsor	Reference	Trial ID
T-Vec	HSV-1	intratumoral	GM-CSF	Approved	Melanoma	Amgen	[12]	NCT00769704
G47Δ	HSV-1	intratumoral	lacZ	II	Glioblastoma	–	[144]	UMIN000015995
G207	HSV-1	intratumoral	lacZ	I/II	Astrocytoma glioblastoma	MediGene	[145]	NCT00028158
JX-594	Vaccinia virus	intratumoral	GM-CSF, lacZ	III	HCC	Sillajen	[146]	NCT02562755
CG0070	Adenovirus	intravesical	GM-CSF	III	Bladder cancer	CG Oncology	[147]	NCT01438112
Reolysin	Reovirus	intravenous	–	III	Head and neck cancer	Oncolytics Biotech	[148, 149]	NCT01166542
GL-ONC1	Vaccinia virus	intraperitoneal	RUC-GFP	II	Peritoneal carcinomatosis	Genelux GmbH	[150]	NCT01443260
CVA21	Coxsackievirus A21	intratumoral	–	II	Malignant melanoma	Viralytics	[151]	NCT01227551
VSV-IFNβ-NIS	VSV	intratumoral	IFNβ, NIS	I	Malignant solid tumor	Vyriad	[152]	NCT02923466
DNX-2401	Adenovirus	intratumoral	–	I	Brain tumor	DNAtrix	[153]	NCT00805376
PVSRIPO	Poliovirus/ rhinovirus	intratumoral	–	I	Malignant glioma	Istari Oncology	[154]	NCT01491893
TG6002	Vaccinia virus	Intrahepatic arterial	FCU1	I/II	Colorectal neoplasms	Transgene	[155]	NCT04194034
MV-NIS	Measles virus	intravenous	NIS	II	Multiple myeloma	University of Arkansas	[156]	NCT02192775

HSV-1 Herpes simplex virus, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *IFNβ* interferonβ, *NIS* human thyroidal sodium iodide symporter, *VSV* vesicular stomatitis virus, *RUC-GFP* light-emitting fusion protein Renilla luciferase-Aequorea green fluorescent protein, *FCU1* fusion suicide gene, *HCC* hepatocellular carcinoma.

and the last reason, when CAR T cells face tumor cells, there is a high risk to undergo exhaustion phenotype, which eventually paralyzes their cytotoxic activity and also diminishes their life span [48].

HOW DOES OV CONVERT A COLD TUMOR TO A HOT TUMOR?

OV-mediated local inflammation

There is an accumulating body of studies suggesting that one of the mechanisms through which OVs could induce antitumor activity is being mediated by enforcing the production of type I interferons (IFNs) in TME [49]. Effector T cells might receive three types of signaling pathways in order to be activated, which are as follows: a signal transmitted upon the interaction of T-cell receptor (TCR) with antigen (Signal 1), a signal mediated through the engagement of co-stimulatory molecules with their ligands expressed on antigen-presenting cells (APCs) (Signal 2), and finally, a signal that is the representative of the presence of pro-inflammatory cytokines or mediators (Signal 3). The second and third generations of CAR T cells have the ability of producing signals 1 and 2. However, signal 3 is recapitulated during the ex vivo expansion via exposing CAR T cells to cytokines and may be additionally assisted either by modifying the ability of CAR T cells to not only produce their own cytokines, but also to deliver a series of cytokines simultaneously as an adoptive cellular therapy [50]. Interestingly, type I IFNs was found to have the ability of stimulating Signal 3 in effector T cells, so if the infused OVs at TME could enhance the production of such cytokine, it could be concluded that OVs not only have the potential of reinforcing the cytotoxic effects of CAR T cells on the tumor milieu, but they could also ameliorate the safety of this immune therapy approach. Moreover, it was indicated that type I IFNs could potentiate the cytolytic function of effector T cells, enhance their clonal proliferation capacity, and more importantly increase the differentiation of these cells to memory cells [50, 51]. Accordingly, one of the best examples of the impact of type I IFNs on mediating antitumor immunity is IFNβ that could facilitate dendritic cells (DCs)-mediated antigen cross-presentation and also disrupt tumor-associated microenvironment by suppressing the activities of both regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) [52]. Briefly, through producing type I INFs and converting the tumor microenvironment from a “cold” status to a “hot” one, it can be said that OVs could facilitate the infiltration, stimulation, and proliferation of CAR T cells [53]. The positive impact of type I IFNs on the efficacy of CAR T cells was well-studied in several preclinical studies. However, it has been claimed that the upregulation of IFNβ and consequently its interaction with interferon receptors (IFNRs) expressed on second-generation of CAR T cells with, 4-1BB as a co-stimulatory molecule, could stimulate TNF receptor-associated factor 2 (TRAF2) as well as its downstream signaling pathway [54].

OV-induced immunogenic cell death

OVs could also increase the release of internal damage-associated molecular patterns (DAMPs), which is an indicator of immunogenic cell death (ICD) [55]. Necrosis, as a type of nonprogrammed cell death, is mostly mediated through pro-inflammatory cytokines. Moreover, necrosis is mostly determined by the release of cell components via a ruptured plasma membrane as well as DAMPs, including heat shock proteins (hsp70 & hsp90) [56], HMGB1, IL-1a, IL-33, ATP, mRNA, genomic DNA, and endoplasmic reticulum (ER) sessile proteins [57]. While the programmed cell death (PCD) is known as a tolerogenic process, some types of PCD such as necroptosis and pyroptosis can also lead to the production of DAMPs. Thereafter, the produced DAMPs serve as either “eat-me” or “danger” signals to immune cells. In this regard, one of the produced DAMPs is cell membrane-associated calreticulin (ecto-CRT), which can be

Table 2. List of current clinical trials assessing CAR T cells in solid tumors.

Target	Cancer type	Signaling domain	Study phase	Dosage	Outcome	Patient number	Route	Sponsor	Year	Reference	Trial ID
α-FR	Ovarian cancer	ζ	I	0.3–5 × 10^{10} Cells	NR	14	I.V	Ovarian Cancer Research Fund	2006	[45]	–
GD2	Neuroblastoma	ζ	I	1.2×10^7 −1 × 10^8cells/m^2	CR 27% (3/11)	11	I.V	Baylor College of Medicine	2011	[157]	NCT00085930
CAIX	Renal cell carcinoma	ζ	I	2×10^7–2 × 10^9	NR	12	I.V	Dutch Cancer Foundation	2013	[47]	–
CEA	Liver metastases	ζ -CD28	I	1×10^8–1 × 10^{10}/kg	PD 83% (5/6) SD 17% (1/6)	6	Hepatic artery infusion	Roger Williams Medical Center	2013	[158]	NCT01373047
CEA	Colorectal cancer	ζ -CD28	I	1×10^8–1 × 10^{10}/kg	SD 70% (7/10) CR 20% (2/10)	10	I.V	Roger Williams Medical Center	2016	[159]	NCT00673322
EGFR	Non-small cell lung cancer	ζ -4-1BB	I/II	0.97×10^7 cells/kg	PR 18% (2/11) SD 45% (5/11)	11	I.V	Chinese PLA General Hospital	2016	[160]	NCT01869166
HER2	Glioblastoma	ζ -CD28	I	1×10^6–1 × 10^8 cells/m2	PR 7% (1/15) SD 27% (4/15)	15	I.V	Baylor College of Medicine	2017	[161]	NCT01109095
HER2	Biliary tract cancer Pancreatic cancer	ζ -4-1BB	I/II	2.1×10^6 cells/kg	PR 9% (1/11) SD 45% (5/11)	11	I.V	Chinese PLA General Hospital	2018	[162]	NCT01935843
EGFRvIII	Glioblastoma	ζ -CD28 + 4-1BB	I	1.75–5 × 10^8 cells	NR	18	I.V	University of Pennsylvania	2019	[163]	NCT02209376
CEA	Liver metastases	ζ -CD28	I	1×10^8–1 × 10^{10}/kg	CR 16% (1/6)	6	Hepatic artery infusion	Roger Williams Medical Center	2019	[164]	NCT02416466

NR not reported, *α-FR* α-folate receptor, *GD2* ganglioside GD2, *CAIX* carbonic anhydrase IX, *CEA* carcinoma embryonic antigen, *EGFR* epidermal growth factor receptor, *HER2* human epidermal growth factor receptor 2, *EGFRvIII* epidermal growth factor receptor variant III.

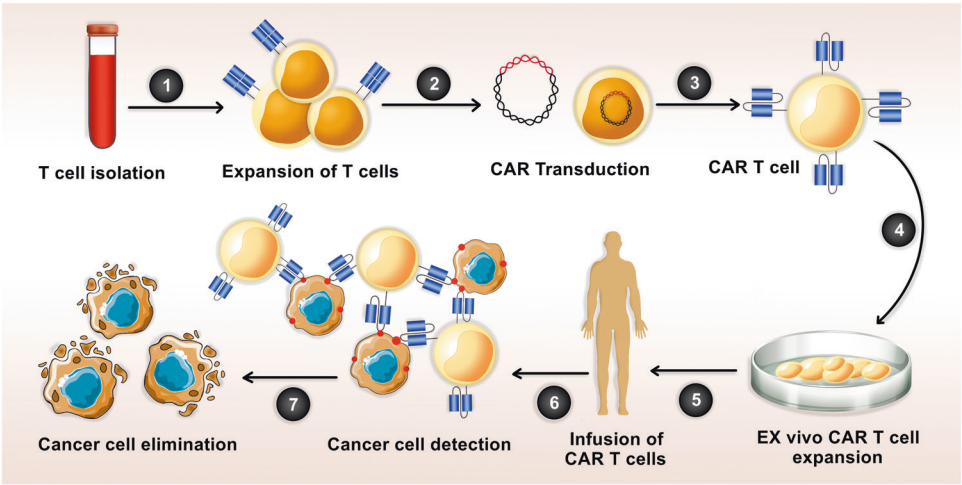

Fig. 1 CAR T-cell application in the treatment of solid tumors. Isolation of T lymphocytes from the patient's peripheral blood; (1) insertion of CARs at the TCR gene locus; (2) Production of cells that express CAR; (3) expansion and proliferation of CAR T cells in vitro; (4) infusion of expanded CAR T cells into the patient's bloodstream; (5) The lower efficacy of CAR T cells in eliminating tumor cells is due to the heterogeneous antigen expression and the induction of immunosuppressive condition in tumor microenvironment (TME).

detected by both migratory and resident scavenging macrophages as well as Batf3+ DCs as tumor-associated antigens (TAA) [58]. Indeed, the identification of ecto-CRT by macrophages and DCs propagates MyD88-NFκB/JAK-STAT signaling cascade, which in turn, increases the expressions of IFNα and β. Once IFN gradients produced, the function of effector T cells would be potentiated, and the resident DCs would be then matured, which is a process enhancing the cross-priming capacity of DCs [59]. OVs also induce ER stress within tumor cells, which consequently elevates the intracellular level of reactive oxygen species (ROS) and increases protein kinase RNA-like endoplasmic reticulum kinase (PERK)-dependent ICD and mitochondrial apoptosis [60]. In the absence of any defective HLA expression or IFN cascade, the remained type I IFNs also increase the expression of class I MHC on either OV-infected or uninfected tumor cells, which consequently potentiates non-CAR based adaptive cell therapy (ACT) [61]. Moreover, immunotherapy with CAR T-cell might overcome a pitfall in those cancer patients, whose tumor cells lost MHC expression in those who are not sensitive to IFNs. As well, T cells can directly identify both DAMPs and PAMPs. For example, the interaction between HMGB1 and RAGE (which is a molecule expressed on effector T cells) could lead to both stimulation and clonal expansion of effector T cells [62, 63].

Up to now, different in vivo and in vitro experiments have investigated the existence of synergistic effects between CAR T cells and OVs, and particularly in solid tumors (Table 3). It was generally assumed that if OVs would be engineered in a way to enforce the expression of some immunomodulatory molecules simultaneously, they may then produce stronger synergistic effects on CAR T cells. However, the results showed some completely different results, as nonengineered OVs made better partnership with CAR T cells. For example, the success of the attenuated herpes virus (HSV1716) was shown in enhancing both expansion and proliferation of GD2-redirected CAR T cell in GD2 expressed cell lines of rhabdomyosarcoma and melanoma cell lines [64]. It should be noted that, in this study, GD2 positive melanoma cells mainly exhibited a resistance phenotype to CAR T cell; however, the companionship of HSV1716 then enhanced the sensitivity of the cells to CAR T-cell immunotherapy. Oncolytic strain of HSV was also found to be capable of promoting cytotoxic function of EGFR-directed CAR NK cells in breast-cancer-derived MDA-MB-231 cells [65]. In the breast cancer brain metastases (BCBMs) model, the dual treatment with the oncolytic strains of HSV and CAR NK cells through the intratumoral inoculation have significantly enhanced the overall survival of tumor-bearing mice compared to monotherapies [65].

The intratumoral administration of oncolytic vesicular stomatitis virus (VSV) in B16.OVA murine melanoma model was indicated to be associated with an elevation in the number of CD8-positive T cells, which consequently improved the median survival of mice to 50% within one month. On the other hand, the median survival of untreated mice and those exposed to heat-inactivated oncolytic VSV was only 20 days. To increase the efficacy of this treatment, the researchers combined the systemic infusion of OT-I T cells with oncolytic VSV treatment. As a result, they found that this approach led to a potent antitumor response and the improved median survival approximately up to 70% within 50 days, as compared to monotherapy. Similarly, intratumoral administration of ex vivo activated OT-I T cells combined along with oncolytic adenovirus resulted in the accumulation of endogenous cytotoxic CD8-positive T cells, which consequently prevented tumor relapse [66]. Accordingly, the combination of adoptive T-cell immunotherapy with oncolytic virotherapy seems to be a promising strategy, especially in immunocompetent murine models. These findings suggested that both -cell and OVs therapies could lead to tumor repression, either as monotherapy or in combination with modality (Table 3).

Reversing TME immunosuppressive signals

In the immunosuppressive TME, cancer cells in line with cancer-associated fibroblasts and stromal cells mostly attenuate the induced antitumor immunity by producing and recruiting of a wide range of soluble and insoluble immunosuppressive molecules and cells. [67]. Cancer cells mainly modify TME by the following process: high VEGF production; anti-inflammatory cytokines; death ligands (such as FasL, PD-1, and TRAIL); nonclassical HLA class I; and several metabolites such as RNS, IDO, and NO [68]. These immunosuppressive components cannot only suppress the induced antitumor immunity, but they can also stimulate the stroma cells and expedite tumor development. Moreover, tumor-associated macrophages (TAMs), regulatory T cells, and MDSCs by production of various immunosuppressive molecules such as arginase I, IDO, ROS, IL-10, TGF-β, and PD-L1 could promote angiogenesis, tumor growth, and metastasis [69–71]. Herein, the main question is how OVs can reverse immunosuppressive TME and also promote the antitumor effect of (CAR) T cells. While OVs were originally developed as cytolytic

Table 3. Preclinical studies combining oncolytic viruses with CAR T cells.

Author/year	Oncolytic agent	Tumor	CAR target	Type of modification	CAR signaling domain	Dose	Outcomes	Ref
Nishio (2014)	Onc.Ad-Rantes/IL-15	Neuroblastoma	Ganglioside GD2	OV armed-cytokines	CD28 + OX40	1×10^7 CART1 × 10^9 Onc.Ad	Tumor growth↓ Overall survival↑	[165]
Wang (2014)	EphA2-TEA-VV	Lung cancer	HER2	OV expression of BiTE	CD3ζ + CD28	1×10^7 CART1 × 10^9 Onc.vv	Tumor growth↓ Survival↑	[116]
Rosewell (2017)	CAdVEC-IL12p70/aPDL1	Head and neck squamous cell carcinoma	HER2	OV armed- IL-12-PD-L1 blocking mini-antibody	CD3ζ + CD28	1×10^6 CART1 × 10^8 Onc.Ad	Tumor growth↓ Survival↑ Metastasis↓	[166]
Tanoue (2017)	CAdVEC-aPDL1	Prostate, squamous cell carcinoma	Human epidermal growth factor 2 (HER2)	OV armed-PD-L1 blocking mini-antibody	CD3ζ + CD28	1×10^6 CART1 × 10^7 Onc.Ad	Tumor volume↓ Survival↑	[167]
Wing (2018)	Onc.Ad-EGFR BiTE	Pancreatic ductal carcinoma/colorectal carcinoma	Folate receptor alpha (FR-a)	OV expression of BiTE	CD3ζ + ICOS	1×10^7 CART1 × 10^9 Onc.Ad	Tumor growth↓ Survival↑	[123]
Watanabe (2018)	Onc.Ad-TNFa/IL-2	Pancreatic ductal carcinoma	Mesothelin (meso)	OV armed-cytokines	CD3ζ + 4-1BB	5×10^6 CAR T -1×10^9 Onc.Ad	Tumor growth↓ Survival↑ Metastasis↓	[107]
Moon (2018)	VV.CXCL-11	Lung cancer	Mesothelin (meso)	OV armed-chemokines	CD3ζ + 4-1BB	1×10^7 CART1 $\times 10^{8}$ $^{VV.CXCL11}$	Tumor volume↓ Survival↑	[110]
Park (2019)	OV19t	Breast cancer	CD19	OV-delivery of target antigen	CD3ζ + 4-1BB	5×10^6 CART1 × 10^7ov19t	Tumor volume↓ Survival↑	[129]
Porter (2020)	CAdTrio	PDA, squamoua cell carcinoma	HER2	OV expression of BiTE	CD3ζ + CD28	1×10^6 CART1 × 10^8CAdTrio	Tumor volume↓ Survival↑	[121]
Li (2020)	rAd.sT	Breast cancer	Mesothelin (meso)	OV armed- sTGFβRIIFc	CD3ζ + CD28 + 4-1BB	1×10^7 meso-CAR T 2.5×10^{10} rAd.sT	Tumor volume Tumor Weight Metastasis↓	[79]
Aalipour (2020)	mCD19VV	Melanoma	CD19	OV-delivery of target antigen	CD3ζ + CD28	1×10^7 CART1 × 10^8 mCD19VV	Tumor volume↓ Survival↑	[127]
Tang (2020)	AdC68-TMC-tCD19	Liver cancer Hepatocellular carcinoma	CD19	OV-delivery of target antigen	CD3ζ + CD28 + 4-1BB	4×10^6 CAR T 8×10^6 AdC68-TMC-tCD19	Tumor volume↓ Survival↑	[128]

TEA-VVs T-cell engager armed vaccinia Virus, *CAdVEC-aPDL1* oncolytic adenovirus (Onc.Ad) with a helper-dependent Ad (HDAd) that expresses a PD-L1 blocking mini-antibody, *rAd.sT* transforming growth factor β signaling-targeted oncolytic adenovirus, *sTGFβRIIFc* soluble TGFβ receptor, *VV.CXCL-11* oncolytic vaccinia virus (VV) engineered to produce CXCL-11, *CAdTrio* oncolytic-helper binary adenovirus (CAdDuo) encoding an IL-12 and PD-L1Ab, *mCD19VV* mCD19 oncolytic vaccinia viruses, *OV19t* oncolytic vaccinia virus coding truncated CD19.

agents, currently, they may also exert some pleotropic effects on TME [72]. The roles of OVs in the stimulation of local inflammation and ICD were discussed in previous sections. The combined production of pro-inflammatory cytokines, PAMPs, and DAMPs triggered by OVs in the tumor milieu was indicated to result in substantial engagement and stimulation of immune cells, particularly central DCs, which are known as the main members of both innate and adaptive arms of immune system [72]. Through uptaking OVs-infected tumor cells and processing their TAAs, both plasmacytoid DCs and human monocyte-derived DCs turn into mature cells and then gain the potential of being involved in antigen cross-presentation to the cytotoxic T cells [72–75]. Subsequently, the activated CD8+ T cells produce perforin and granzymes for destroying tumor cells expressing the specific tumor antigens. T-cell cross-priming by DCs have also been reported following both reovirus and parvovirus induced tumor cell oncolysis [76]. Another study have previously described that infection of tumor cells by different OVs elicits the delivery of TAAs within TME, which consequently facilitates the identification of TAA-loaded tumor cells by CD4 positive T cells [77]. On other hand, OVs also target cancer stromal cells, including pericytes, cancer-associated fibroblast (CAF), and endothelial cells, hence simultaneously destroying the complex structure of TME and increasing tumor penetration rate and availability for immune infiltration [78]. Therefore, OV-induced antitumor immune response was shown to counteract the immunosuppressive TME [79, 80]

Blunting tumor relapse

Despite performing the maximal initial therapy, tumor relapse still remains a major clinical challenge. Even with repeated surgery, reirradiation, and chemotherapy, most types of tumors invariably recur yet. It has been reported that emergent recurrent tumors gain a phenotype distinct from that of their originated primary tumors [81]. Correspondingly, this new phenotype facilitates evasion from host-derived antitumor immune response that is induced by the advancement from minimal residual disease (MRD) to the dynamically growing tumor relapse [82].

In recent years, oncolytic adenoviruses have been identified as appealing therapeutic modalities, as they can efficiently destroy cancer stem cells (CSCs) as well as eliciting cell death through various mechanisms such as upregulating the expression of some toxic proteins, direct lysis, T-cell-mediated immunity, and the induction of cytokines [83]. GBM stem-like cells, as a group of cancer cells with stemness capability, are mostly accounted for tumor relapse. In the study by Jahan et al., the therapeutic effect of oHSV (oHSV-TRAIL) on Temozolomide (TMZ) resistant patient-derived GBM stem cells was evaluated [84]. In this regard, it was reported that oHSV-TRAIL regulated DNA damage response pathways, cell survival, and MAPK signaling axis in both primary and recurrent TMZ-resistant GBM stem cells [84]. Moreover, it was demonstrated that oHSV-TRAIL could effectively suppress tumor progression and consequently increase overall survival of animal bearing TMZ-insensitive recurrent intracerebral GBM stem-like cells through the strong targeted initiation of apoptosis, thereby, leading to tumor eradication in 40% of the treated mice [84].

In another study, the efficacy and mechanisms of action of the DNX-2401 (Delta-24-RGD; tasadenoturev) were evaluated in patients with recurrent malignant glioma [85]. Moreover, it has been reported that DNX-2401 could dramatically prolong the survival of patients with recurrent malignant glioma possibly through increasing the presence of cytotoxic CD8-positive and T-bet-positive cells as well as the downregulation of transmembrane immunoglobulin mucin-3 (TIM-3) [85]. In addition, another mechanism leading to tumor relapse is the induction of exhaustion phenotype in T cells [86]. Synergisms of TIM-3 and PD-1, which are both inhibitory receptors, could also regulate exhausted T cells [86] and collaborate in triggering $CD8^+$ T-cell suppression [87, 88]. While tumor infection with DNX-2401 does not significantly modify PD-1 expression, performing a treatment with DNX-2401 downregulated TIM-3 represents that DNX-2401 might moderately overwhelm T-cell exhaustion in immunosuppressive tumor settings as well as inhibiting tumor relapse [85].

Different studies have previously suggested the essential role of TGF-β in sustaining stemness of both GBM stem cells and GBM recurrence. In the study conducted by Esaki et al., the synergistic effects of TGF-β inhibitors and oncolytic herpes simplex virus (oHSV) on the treatment of recurrent GBM, were evaluated [89]. It was indicated that, signaling of TGF-β induces phenotype resistance against radiation, immune-suppression invasion/migration, and angiogenesis in GBM [90].

The therapeutic values of TGFβR kinases inhibition and oHSV have been tested in patient-derived recurrent GBM models [89]. Interestingly, while these GBM stem cells have shown resistant phenotype against temozolomide, they were simultaneously sensitive to both MG18L and oHSVs G47Δ. In particular, the use of oHSV-plus-TGFβR inhibitor was successful in eliminating the number of recurrent GBM stem cells and also in reducing the risk of disease relapse through suppressing of both JNK and APK signaling pathways [89].

It was found that cancer cells producing the CAR-targeted antigen can evade adaptive T-cell therapy by losing the antigen's expression, particularly when targeting non-oncogenic or non-driving molecules [91]. Cancer cells by applying various mechanisms, including deletion or downregulation of target antigens, antigen mutation, and selective survival of antigen-negative cancer cells, are escaping from immune recognition [92]. The decreased surface expression of CD22 on B-cell ALL cells has been recognized as a relapse mechanism in response to CD22-CAR immunotherapy [93]. Following the CAR T-cell therapy, OV therapy by the stimulation of endogenous immune effector cells and non-CAR T cells can synergistically target tumor cells and also prevent antigen-negative relapse [94]. In this regard, it has been suggested that the intratumoral delivery of an oncolytic virus engineered to recruit immune cells might favorably modify the immunosuppressive TME in the inoculated lesions, enhance CD8+ T-cell infiltration, and suppress antigen-negative relapse [80, 94].

THE MODIFIED OVS WITH PAYLOADS FOR AUGMENTING THE FUNCTION OF CAR

Re-engineering OVs with some anticancer agents for regulating TME showed some promising results in several clinical investigations. More specifically, it was found that several of them will be extremely appropriate for combining CAR T-cell therapy with ACT. After the infection of tumor cells with these OVs, the expression profile of cytokines or chemokines in TME would change, an event that in turn converts the hostile environment to a more accommodating place not only for the entrance of CAR T cell, but also for their stimulation, expansion, and persistence. Alternatively, these recombinant OVs may promote epitope spreading, endogenous antitumor immune response, and eventually result in an effective combined antitumor response (Fig. 2).

The ameliorating impact of OVs in CAR T-cell function through cytokines delivery

In recent years, cytokines have increasingly identified as an important therapeutic agent for the treatment of patients at the advanced stages of cancer or those with metastatic cancer [95]. The local carriage of cytokines by OVs seems to be a more appropriate and safer strategy to be used along with CAR T-cell therapy, as compared to so-called "armored CARs" [8]. Up to now, various cytokine-armed OVs have been designed such as those that could produce TNFα [96]. Type I IFNs [97], IL-2 [98], IL-18 [99], IL-4 [100], IL-15 [101], and IL-12 [102]. Apart from pro-inflammatory functions, a large number of these mediators (most

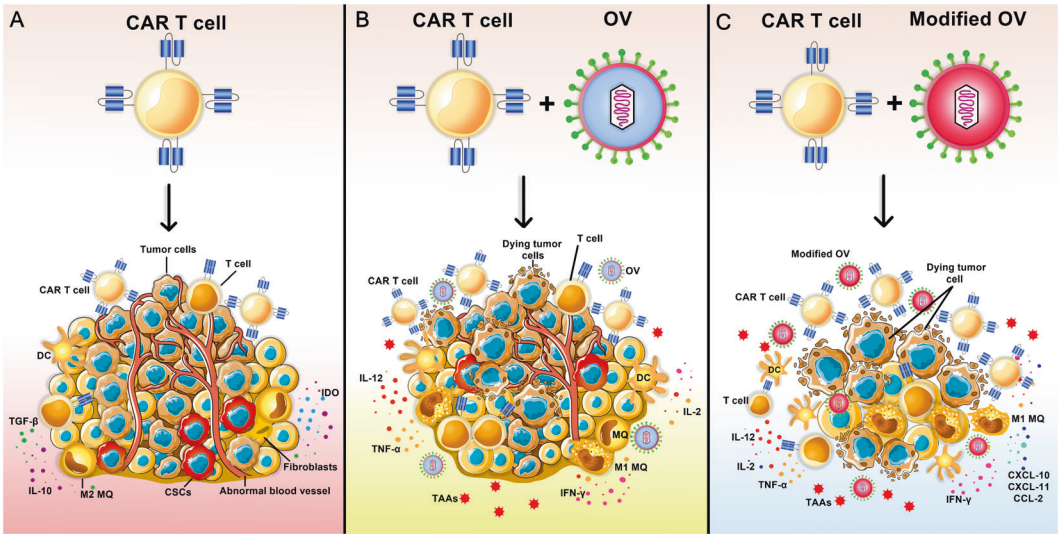

Fig. 2 Oncolytic virus and CAR T-cells combination therapy in solid cancers. A The failure of CAR T cells in the treatment of solid tumors is due to the induction of immunosuppressive TME and low penetration rate. **B** Treatment of cancer cells with oncolytic viruses before the administration of CAR T cells may lead to immunogenic cell death, tumor debunking, and converted cold tumor to hot tumor. **C** The genetically engineering of oncolytic viruses with payloads (Cytokines, Chemokines, BiTEs, and…) may promote effector T cells and CARs functions. The combination of CAR T cells and oncolytic viruses expressing immune checkpoint inhibitors, chemokines, cytokines, and BiTEs was coupled with promising therapeutic outcomes in preclinical investigations. DC dendritic cell, BiTEs bi-specific T-cell engagers, TGF transforming growth factor, IL-10 interleukin 10, CSCs cancer stem cells, IDO indoleamine 2 3-dioxygenase, TAAs tumor-associated antigens, MQ macrophage, TNF tumor necrosis factor, CXCL-10 C-X-C motif chemokine ligand 10, CCL-2 C-C motif Chemokine Ligand 2.

notably type I IFNs and TNFα) might represent a direct toxicity on bystander cancer cells based on their intrinsic susceptibility [102]. In the xenograft model of glioblastoma, it has been reported that an oncolytic HSV equipped with IL-12 and IL-4 successfully induced antitumor immune response [103]. Accordingly, similar results were also obtained from a study that evaluated the therapeutic potential of oncolytic adenovirus dually equipped with IL-18 and IL-12 in the B16-F10 murine model of melanoma [104]. Likewise, arming recombinant Newcastle disease virus (rNDV) with IL-2 along with TRAIL (rNDV-IL-2-TRAIL) through regulating apoptosis in melanoma and hepatocellular carcinoma cells and promoting the proliferative capacity of both CD4 and CD8-positive T cells, remarkably improved intrinsic antineoplastic of rNDV [105].

In another study performed by Patel et al., the intratumoral injection of an IFNβ armed VSV was found to be related to tumor regression, the improved disease control, and the complete tumor eradication in more than 30% of mice in the syngeneic lung cancer model [106]. So far, few preclinical and clinical studies have been conducted on investigating the synergistic effects of CAR T-cell therapy and cytokine-armed OVs. As an example in this field, the study of Watanabe et al., can be named, in which an engineered adenoviral OV that could produce either TNFα or IL-2 was used combined with a mesothelin directed 4-1BB-containing the second-generation CAR in Pancreatic ductal adenocarcinoma (PDA) model [107]. Herein, the combination therapy was demonstrated to promote the efficacy of CAR T cell, as the produced TNFα and IL-2 both could counteract with the cancer metastasis development. Furthermore, the combination therapy was observed to be related to the deviation of macrophages to an M1 subset, which improved maturation of dendritic cell and the local recruitment of both adoptively transferred donor CAR T cells and non-CAR host T cells through the secretion of TNFα-inducible chemokines such as CXCL-10, CCL-2, and CCL-5 [107].

In a similar approach, Hurton et al., generated CAR T cells with the preserved T-memory stem cells (TSCM) potential utilizing the Sleeping Beauty platform (SBP) [108]. Given the confirmed role of IL-15 in the regulation of T-cell memory, it fused a membrane bound IL-15/IL-15Rα molecules to CAR T cell (mbIL15-CAR T cells). This CAR T cell could exhibit both cis- and trans-mediated IL-15-activations that increase both in vitro and in vivo proliferation and persistence, respectively [108]. Moreover, although mbIL15-CAR T cells elevated the formation of CD45RO CCR7 + CD95 + TSCM memory subset, they were found to be affected by several stages of the ex vivo activation [108]. Altogether, these results demonstrated that tethered IL-15 could improve antitumor immune response through inducing a stem-cell memory phenotype in tumor-specific T cells.

In TME, the TGF-β signaling pathway is considered as an essential signal for inducing immunosuppression. In the study by Li et al., [79], the efficacies of the combination of rAd.sT, a TGF-β signaling-targeted oncolytic adenovirus, and CAR T-cell therapy were evaluated in a triple-negative breast cancer cells (MDA-MB-231). As a result, they reported that rAd.sT could directly lyse malignant cells and have considerable antitumor effects at early stages; however, the antitumor effect reduced at the advanced stage. Nevertheless, CAR T-cell immunotherapy showed the most robust and long-term antitumor response. Notably, the combination therapy of CAR T with cells with rAd.sT also generated the strongest antitumor immune responses and therapeutic effects [79], (Table 3).

The effect of OVs on CAR T cells through chemokine delivery

Given the role of several cytokines, chemokines and adhesion molecules in attracting the endogenous CTLs into tumor milieu, it could be suggested that if an OV deliver these chemokines, they could be then recruited to overcome the challenges of CAR T cells homing via increasing their infiltration to tumor site [109].

CAR T cells that are adoptively transferred usually overexpress CXCR3 and affording from signal 2 (and signal 3 in the fourth generation) originated from the CAR endodomain [110]. However, for an efficient trafficking and tumor homing, they would be estimated yet to demonstrate if they need an inflamed tumor milieu with sufficient chemoattractant chemokines concentrations. Oncolytic viruses, which can generally stimulate the type I IFNs local secretion, have the potential of being equipped with corresponding chemokines, and then act as an efficient partner in the combination immunotherapy applying CAR T cells [111]. In

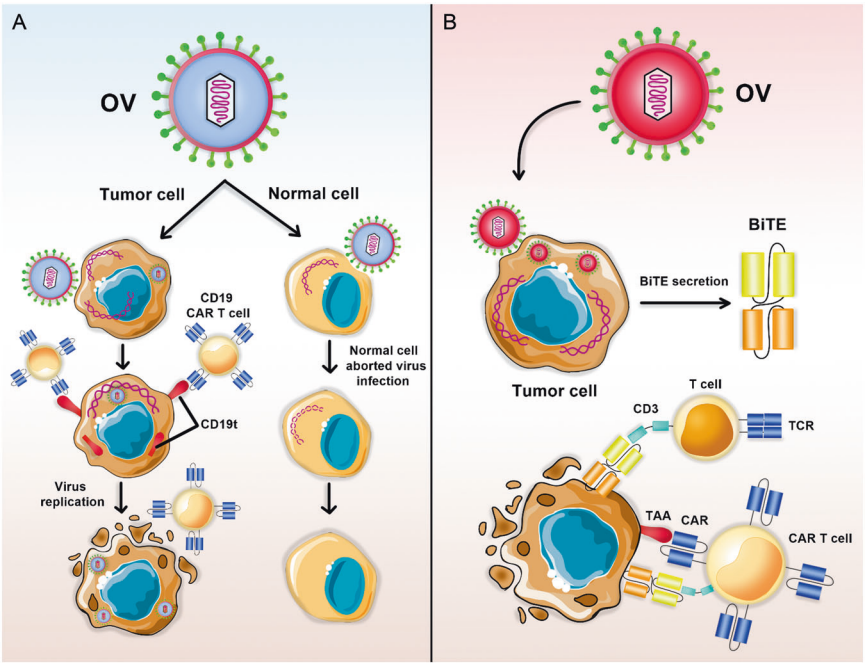

Fig. 3 Oncolytic virus-mediated delivery of tumor-selective surface antigens. A The administration of oncolytic vaccinia virus expressing truncated CD19 (CD19t) molecule not only increased the expression of CD19t in tumor cells, but also enhanced the antitumor activity of CD19-CAR T cells. **B** Secretion of bi-specific T-cell engagers (BiTEs) by oncolytic virus induce tumor cell death. BiTEs are artificial bispecific monoclonal antibodies constructing of two fused single-chain variable (ScFv) domain of antibodies (anti-CD3 linked to an anti- TAA). While normal cells can abort oncolytic virus infection, OVs engineered to secrete BiTEs can infect and replicate in tumor cells. Oncolytic virus-driven TAAs expression synergizes with BiTE and CAR T-cell immunotherapies.

tumor immunotherapy, OVs are genetically modified to generate chemokines, in order to reinforce the infiltration of cytotoxic T cells, DC, macrophages, or other immune cells into the tumor milieu [112]. It has been demonstrated that the direct intratumoral administration of a CXCL-11 armed vaccinia OV strain leads to augmentation of the infiltration of tumor-specific cytotoxic T cells in the syngeneic mouse mesothelioma model [113]. Moreover, significant downregulation of immune suppressive cytokines and chemokines such as CCL-22 (a Treg chemoattractant), TGFβ, COX2, along with a simultaneous overexpression perforin and granzyme B, were shown in this model [113]. A second study performed by Moon EK et al. have evaluated the synergistic effects of CXCL-11 and mesothelin-redirected CAR T cells on mesothelioma patients and also on murine model [110]. Accordingly, in this study, CXCL-11 was transferred to the targeted tumor tissue either via subcutaneous injection of a vaccinia virus armed with CXCL-11 (VV.CXCL-11) or via an upregulation in adoptively transferred T cells transfected with a lentiviral transgene cassette, which expressed a 4-1BB containing both anti-mesothelin CAR T cell and CXCL-11 [110]. However, the use of both approaches was shown to have the ability of elevating CXCL-11 expression in TME, only VV.CXCL11could promote the antitumor efficacy following the adoptively transferred T cells using mesothelin-redirected CARs [110].

Expression of bispecific T-cell engagers (BiTE) by OVs

The capability of OV agents in restoring the tumor milieu and stimulating the augmented adaptive antitumor immune response have been revolutionized by combining them with some engineered molecules, in order to engage TCR complex of effector T cells. Correspondingly, these engineered molecules could simultaneously interact with CD3ε from TCR complex and TAA [114] (Fig. 3). The efficacy of Blinatumomab, as a preliminary BiTE that could target both CD3 and CD19 has been previously tested in clinical trial for the treatment of refractory B-cell acute lymphoblastic leukemia (B-ALL) [115]. As well, the potential synergies between BiTEs and OVs have been explored by various study groups. In this regard, Wang et al. designed a novel T-cell Engager Armed Vaccinia Virus (TEA-VVs) with the ability of releasing bispecific antibodies that interacting with either EphA2 (cell surface Antigen) or CD3 [116]. Thus, the infection with OV could stimulate T-cell-mediated bystander eradication of non-infected cancer cells. Additionally, when this oncolytic agent is used along with a HER2-redirected CAR, the reduction in the survival of triple positive HER2/ EphA2/ A549 tumor cells is reinforced more remarkably, which is an indicative of the efficacy of this approach in resolving cold tumor heterogeneity as well as hindering CAR-mediated antigen escape [116]. Actually, given the confirmed capability of BiTE in redirecting adaptive transferred T cells and CAR as well as that of OVs in regulating the inflammatory responses in TME, it seems that this combinatorial approach would probably bring new insight on the treatment of solid tumors [117, 118]. The efficacy of combinational immunotherapy was later intensified in the results of a previous study that indicated the proliferative ability of TEA-VV in HER2-redirected CAR T cells, which suggested that CAR could be used as a protective delivery carriage for TEA-VV, while sheltering it from host exclusion [119]. Independently, this novel strategy could be utilized in the treatment of tumor stroma and at any occasion in which T-cell-mediated immune response is banned due to either immunosuppressive conditions or physical restrictions. Yu et al. in their study evaluated the potential of TEA-VV expressing BiTE that targeted fibroblast activation protein (FAP) and murine CD3 in the xenograft murine model of melanoma [119]. As a result, this in vitro investigation showed that upon the existence of murine T cells, mFAP-TEA-VV could stimulate bystander eradication of noninfected FAP+ stromal cells. Moreover, in vivo mFAP-TEA-VV transfection resulted in higher viral titers as well as lower metastatic tumor burden [119]. In a similar study, it has been reported that the use of bispecific T-cell Engager against FAP

Table 4. Ongoing clinical trials investigating the combination of CAR T cells with OVs in solid tumors.

Oncolytic agent	CAR target	Tumor	Phase	Status	Outcome measures	Sponsor	Trial ID
CAdVEC	HER2	Bladder cancer, head and neck squamous cell carcinoma, cancer of the salivary gland lung cancer, breast cancer gastric cancer, esophageal cancer colorectal cancer, pancreatic adenocarcinoma	I	Not yet recruiting	DLT ORR DCR PFS OS	Baylor College of Medicine	NCT03740256
varicella zoster virus	GD2	Osteosarcoma neuroblastoma	I	Active, not recruiting	DLT ORR	Baylor College of Medicine	NCT01953900

DLT dose limiting toxicity, *ORR* overall response rate, *DCR* disease control rate, *PFS* progression-free survival, *OS* overall survival.

(FBiTE) facilitated binding of FAP+ target tumor cells and CD3+ effector T cells, which eventually resulted in stimulation, proliferation, and cytotoxicity of T cells against FAP-positive cells. As well, FBiTE expression enhanced T cells' infiltration and then diminished the expression of FAP in TME in vivo [120]. Recently, in a study conducted by Porter et al., the improved potency, duration, and breadth of antitumor activity of CAR T cells applying BiTE- checkpoint blockade and cytokine expressing OV, were reported. In this study, CD44 variant 6 (CD44v6) specific BiTE was subjected to binary adenovirus (CAdDuo), which has the ability of expressing both PD-L1Ab and IL-12 to design CAdTrio [121]. Furthermore, it has been indicated that CD44v6 BiTE could increase the sensitivity of CD44v6-expressing tumor cell lines to the cytotoxic effect of HER2-specific CAR T cells. CD44v6 BiTE was also found to reinforce the antitumor activity of HER2-specific CAR T cells in orthotopic $HER2^{-/-}$ $CD44v6^{+}$ and $HER2^{+}$tumors [121].

Similar approaches have been also used related to adenoviral OVs [122]. It has been demonstrated that EGFRBiTE-armed adenovirus (OAd-BiTE) could consequently increase the antitumor activity of CAR T cells at low antigen density. Moreover, when it became evident that heterogeneous FRα expression could result in the induction of resistance against single therapy of CAR T cells in NSG mice bearing SKOV tumors, it has been suggested that probably the use of the second-generation ICOS-armed anti- FRα CAR along with OAd-BiTE might bring some promising results [123].

OV-mediated delivery of tumor-selective surface antigens

Identifying target cells by CAR T cell is highly associated with antigen density, implying that even solid cancers expressing TAAs, may still be unresponsive to therapy [124]. Despite proposing novel strategies for enhancing CAR T-cell function, few studies have been conducted on modifying tumors for an efficient CAR T-cell therapy. Moreover, the lack of targetable surface tumor antigens still remains a major challenge [125, 126]. Therefore, to resolve these problems, a novel approach of OV-mediated delivery of tumor-selective surface antigens has recently been introduced, in order to augment the antitumor activity of CAR T-cell therapy (Fig. 3A). Aalipour et al. engineered a thymidine kinase-disrupted vaccinia virus for the targeted transfer of CD19 to tumor cells. Moreover, an in vitro evaluation verified the promoted the cytotoxic activity of CD19-CAR T cell against two distinct tumor cell lines. Furthermore, this approach significantly diminished tumor growth and improved median survival in the murine models of B16 melanoma compared with antigen-mismatched combinations [127]. In another study, a recombinant adenovirus expressing CD19 tag (AdC68-TMC-tCD19) was constructed, which could label various solid tumors for single anti-CD19-CAR T detection. Correspondingly, this engineered adenovirus could exert the generalized tag expression and then form immunological synapses between CAR T and neoplastic cells. Interestingly, all these tagged animals survived after CAR T injection and tumor growth was also inhibited in the premixed mice model by ~92%. In addition, they combined the oncolysis ability with tumor tagging to construct the replicative AdC68-Sur-E1A-TMC-tCD19. It was reported that an oncolytic tagging system could significantly prolong mice survival and eradicate the established cancers in the mice models [128]. In a similar investigation conducted by Park et al., an oncolytic vaccinia virus was engineered to manifest the truncated CD19 (CD19t) molecule for tumor-selective delivery. Their results demonstrated that intratumoral administration of OV19t consequently upregulated CD19t and promoted tumor inhibition in response to CD19-CAR T-cell infusion [129], (Table 3).

CURRENT CHALLENGES AND FUTURE DIRECTIONS

These preclinical findings evidently highlighted that, while solid tumors are educated at escaping immunotherapies, a

combination of CAR T-cell immunotherapy with OVs can overwhelm these evasion mechanisms. In addition, recent clinical trials implied that combinatorial immunotherapy approaches targeting various aspects will be needed to eliminate tumors [130, 131]. Moreover, the general significance of the OV in attracting T cells to TME has been well established [132, 133]; however, principal questions on the best dosing schedules and delivery routes remained unanswered. In this regard, it is true that intratumoral injection of the virus may deliver a larger number of OVs in the injected tumor lesions, but applying this approach is not suitable in the cases of tumor metastasis or visceral tumors, as the low number of injected OVs could not remarkably counteract with tumor-induced immunosuppressive milieu. So, it seems that systemic delivery may be considered as a solution for this restriction, especially in metastatic lesions; however, this approach may stimulate the production of neutralizing antibodies against circulating OVs, which eventually decrease the efficacy of OVs in repeated administrations. Moreover, systemic or intratumoral delivery of the virus may induce a very different immune response, and timing schedules of the CAR T cells and virus can affect the outcome. Technically, prior to the CAR T-cell infusion, OVs should be delivered in order to alter the immune suppressive TME, impose a lytic effect on cancer cells, and then provide a platform for attracting CAR T cells. The patients' preconditioning should also be considered prior to the therapy. While the inflammatory environment induced by the OV may overrule the necessity of patient's lymphodepletion for promoting CAR T-cell proliferation, lymphodepletion could yet be regarded as an appropriate strategy used to improve virus persistence and replication in TME [134–137]. Of note, oncolytic agents provide a robust oncolytic and inflammatory activities that could increase the release of TAA. Nevertheless, the potential of OV in stimulating an antitumor immunity has still remained elusive. Considering the large number of viral non-self-epitopes following OV therapy, immune responses to the viral antigens may dominate the response in combination with the released tumor neoantigens [138–140]. By remembering this point in mind, finding new approaches, in order to enhance the immunogenicity of cancer antigens and decrease the immunodominance of viral epitopes, is required to increase epitope spreading [141]. Last, but not least, T cells are good candidates to be used in along with OVs. Virus-specific and central memory T cells have been applied as a CAR expression frameworks [142, 143]. These-specific CAR T cells maintain the capability of distinguishing both tumor and virus-infected targets by their chimeric and native receptors, respectively. Consequently, this new generation of T cells could be used as a suitable option in combination with OVs, as the viruses could reinforce the attraction of CAR T cells in TME. However, the main pitfall of this strategy is the rapid clearance of OVs. Hence, the complete success of this combination approach in preclinical and clinical studies performed in future (Table 4) is strictly associated with addressing the current challenges.

REFERENCES

1. Hargadon KM, Johnson CE, Williams CJ. Immune checkpoint blockade therapy for cancer: an overview of FDA-approved immune checkpoint inhibitors. Int Immunopharmacol. 2018;62:29–39.
2. Rossig C, Brenner MK. Genetic modification of T lymphocytes for adoptive immunotherapy. Mol Ther. 2004;10:5–18.
3. Guedan S, Calderon H, Posey AD Jr, Maus MV. Engineering and design of chimeric antigen receptors. Mol Ther Methods Clin Dev. 2019;12:145–56.
4. Stoiber S, Cadilha BL, Benmebarek M-R, Lesch S, Endres S, Kobold S. Limitations in the design of chimeric antigen receptors for cancer therapy. Cells. 2019;8:472.
5. Abken H, Chmielewski M, Hombach AA. Antigen-specific T-cell activation independently of the MHC: chimeric antigen receptor-redirected T cells. Front Immunol. 2013;4:371.
6. Dai H, Wang Y, Lu X, Han W. Chimeric antigen receptors modified T-cells for cancer therapy. J Natl Cancer Inst. 2016;108:1–14.
7. Yu W-L, Hua Z-C. Chimeric antigen receptor T-cell (CAR T) therapy for hematologic and solid malignancies: efficacy and safety—a systematic review with meta-analysis. Cancers (Basel). 2019;11:47.
8. Wang X, Wu Z, Qiu W, Chen P, Xu X, Han W. Programming CAR T cells to enhance anti-tumor efficacy through remodeling of the immune system. Front Med. 2020;14:1–20.
9. Tahmasebi S, Elahi R, Esmaeilzadeh A. Solid tumors challenges and new insights of CAR T cell engineering. Stem Cell Rev Rep. 2019;15:619–36.
10. Abreu TR, Fonseca NA, Gonçalves N, Moreira JN. Current challenges and emerging opportunities of CAR-T cell therapies. J Control Release. 2020;319:246–61.
11. Russell SJ, Barber GN. Oncolytic viruses as antigen-agnostic cancer vaccines. Cancer Cell. 2018;33:599–605.
12. Andtbacka R, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol. 2015;33:2780–8.
13. Chesney J, Puzanov I, Collichio F, Singh P, Milhem MM, Glaspy J, et al. Randomized, open-label phase II study evaluating the efficacy and safety of talimogene laherparepvec in combination with ipilimumab versus ipilimumab alone in patients with advanced, unresectable melanoma. J Clin Oncol. 2018;36:1658.
14. Marelli G, Howells A, Lemoine NR, Wang Y. Oncolytic viral therapy and the immune system: a double-edged sword against cancer. Front Immunol. 2018;9:866.
15. Bommareddy PK, Shettigar M, Kaufman HL. Integrating oncolytic viruses in combination cancer immunotherapy. Nat Rev Immunol. 2018;18:498.
16. Bauzon M, Hermiston T. Armed therapeutic viruses–a disruptive therapy on the horizon of cancer immunotherapy. Front Immunol. 2014;5:74.
17. Twumasi-Boateng K, Pettigrew JL, Kwok YE, Bell JC, Nelson BH. Oncolytic viruses as engineering platforms for combination immunotherapy. Nat Rev Cancer. 2018;18:419–32.
18. Cao G-D, He X-B, Sun Q, Chen S, Wan K, Xu X, et al. The oncolytic virus in cancer diagnosis and treatment. Front Oncol. 2020;10:1–12.
19. Ma J, Ramachandran M, Jin C, Quijano-Rubio C, Martikainen M, Yu D, et al. Characterization of virus-mediated immunogenic cancer cell death and the consequences for oncolytic virus-based immunotherapy of cancer. Cell Death Dis. 2020;11:1–15.
20. Ghaleh HEG, Bolandian M, Dorostkar R, Jafari A, Pour MF. Concise review on optimized methods in production and transduction of lentiviral vectors in order to facilitate immunotherapy and gene therapy. Biomed Pharmacother. 2020;128:110276.
21. Larocca CA, LeBoeuf NR, Silk AW, Kaufman HL. An update on the role of talimogene laherparepvec (T-VEC) in the treatment of melanoma: best practices and future directions. Am J Clin Dermatol. 2020;21:1–12.
22. Packiam VT, Lamm DL, Barocas DA, Trainer A, Fand B, Davis RL, III, et al. An open label, single-arm, phase II multicenter study of the safety and efficacy of CG0070 oncolytic vector regimen in patients with BCG-unresponsive non-muscle-invasive bladder cancer: Interim results. Urol Oncol. 2018;36:440–7.
23. Park SH, Breitbach CJ, Lee J, Park JO, Lim HY, Kang WK, et al. Phase 1b trial of biweekly intravenous Pexa-Vec (JX-594), an oncolytic and immunotherapeutic vaccinia virus in colorectal cancer. Mol Ther. 2015;23:1532–40.
24. Heo J, Breitbach C, Cho M, Hwang T-H, Kim CW, Jeon UB, et al. Phase II trial of Pexa-Vec (pexastimogene devacirepvec; JX-594), an oncolytic and immunotherapeutic vaccinia virus, followed by sorafenib in patients with advanced hepatocellular carcinoma (HCC). Am Soc Clin Oncol. 2013;31:1–15.
25. Galanis E, Markovic SN, Suman VJ, Nuovo GJ, Vile RG, Kottke TJ, et al. Phase II trial of intravenous administration of Reolysin®(Reovirus Serotype-3-dearing Strain) in patients with metastatic melanoma. Mol Ther. 2012;20:1998–2003.
26. Mahalingam D, Goel S, Aparo S, Patel Arora S, Noronha N, Tran H, et al. A phase II study of pelareorep (REOLYSIN®) in combination with gemcitabine for patients with advanced pancreatic adenocarcinoma. Cancers (Basel). 2018;10:160.
27. Todo T, Martuza RL, Rabkin SD, Johnson PA. Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. Proc Natl Acad Sci USA. 2001;98:6396–401.
28. Sugawara K, Iwai M, Yajima S, Tanaka M, Yanagihara K, Seto Y, et al. Efficacy of a third-generation oncolytic herpes virus G47Δ in advanced stage models of human gastric cancer. Mol Ther Oncolytics. 2020;17:205–15.
29. Mondal M, Guo J, He P, Zhou D. Recent advances of oncolytic virus in cancer therapy. Hum Vaccin Immunother. 2020;16:2389–402.
30. Esaki S, Goshima F, Kimura H, Murakami S, Nishiyama Y. Enhanced antitumoral activity of oncolytic herpes simplex virus with gemcitabine using colorectal tumor models. Int J Cancer. 2013;132:1592–601.
31. Trager MH, Geskin LJ, Saenger YM. Oncolytic viruses for the treatment of metastatic melanoma. Curr Treat Options Oncol. 2020;21:1–16.

32. Hashiguchi T, Kajikawa M, Maita N, Takeda M, Kuroki K, Sasaki K, et al. Crystal structure of measles virus hemagglutinin provides insight into effective vaccines. Proc Natl Acad Sci USA. 2007;104:19535–40.
33. Bhattacharjee S, Yadava PK. Measles virus: background and oncolytic virotherapy. Biochem Biophysics Rep. 2018;13:58–62.
34. Russell SJ, Peng K-W, Bell JC. Oncolytic virotherapy. Nat Biotechnol. 2012;30:658.
35. MacKay M, Afshinnekoo E, Rub J, Hassan C, Khunte M, Baskaran N, et al. The therapeutic landscape for cells engineered with chimeric antigen receptors. Nat Biotechnol. 2020;38:233–44.
36. Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 2017;543:113–7.
37. Wang X, Rivière I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. Mol Ther Oncolytics. 2016;3:16015.
38. Ellis J. Silencing and variegation of gammaretrovirus and lentivirus vectors. Hum Gene Ther. 2005;16:1241–6.
39. Von Kalle C, Deichmann A, Schmidt M. Vector integration and tumorigenesis. Hum Gene Ther. 2014;25:475–81.
40. MacLeod DT, Antony J, Martin AJ, Moser RJ, Hekele A, Wetzel KJ, et al. Integration of a CD19 CAR into the TCR alpha chain locus streamlines production of allogeneic gene-edited CAR T cells. Mol Ther. 2017;25:949–61.
41. Habib R, Nagrial A, Micklethwaite K, Gowrishankar K. Chimeric antigen receptors for the tumour microenvironment. Tumor Microenviron. 2020;1263:117–43.
42. Wu L, Wei Q, Brzostek J, Gascoigne NR. Signaling from T cell receptors (TCRs) and chimeric antigen receptors (CARs) on T cells. Cell Mol Immunol. 2020;17:1–13.
43. Pan J, Yang J, Deng B, Zhao X, Zhang X, Lin Y, et al. High efficacy and safety of low-dose CD19-directed CAR-T cell therapy in 51 refractory or relapsed B acute lymphoblastic leukemia patients. Leukemia. 2017;31:2587–93.
44. Moon EK, Wang L-C, Dolfi DV, Wilson CB, Ranganathan R, Sun J, et al. Multifactorial T-cell hypofunction that is reversible can limit the efficacy of chimeric antigen receptor–transduced human T cells in solid tumors. Clin Cancer Res. 2014;20:4262–73.
45. Kershaw MH, Westwood JA, Parker LL, Wang G, Eshhar Z, Mavroukakis SA, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res. 2006;12:6106–15.
46. Xu J, Tian K, Zhang H, Li L, Liu H, Liu J, et al. Chimeric antigen receptor-T cell therapy for solid tumors require new clinical regimens. Expert Rev Anticancer Ther. 2017;17:1099–106.
47. Lamers CH, Klaver Y, Gratama JW, Sleijfer S, Debets R. Treatment of metastatic renal cell carcinoma (mRCC) with CAIX CAR-engineered T-cells—a completed study overview. Biochem Soc Trans. 2016;44:951–9.
48. Li J, Li W, Huang K, Zhang Y, Kupfer G, Zhao Q. Chimeric antigen receptor T cell (CAR-T) immunotherapy for solid tumors: lessons learned and strategies for moving forward. J Hematol Oncol. 2018;11:22.
49. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discov. 2015;14:642–62.
50. DeRenzo C, Gottschalk S. Genetic modification strategies to enhance CAR T cell persistence for patients with solid tumors. Front Immunol. 2019;10:218.
51. Labarta-Bajo L, Nilsen SP, Humphrey G, Schwartz T, Sanders K, Swafford A, et al. Type I IFNs and CD8 T cells increase intestinal barrier permeability after chronic viral infection. J Exp Med. 2020;217:1–19.
52. Zhao Z, Condomines M, van der Stegen SJ, Perna F, Kloss CC, Gunset G, et al. Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. Cancer Cell. 2015;28:415–28.
53. Pikor LA, Bell JC, Diallo J-S. Oncolytic viruses: exploiting cancer's deal with the devil. Trends Cancer. 2015;1:266–77.
54. Benmebarek M-R, Karches CH, Cadilha BL, Lesch S, Endres S, Kobold S. Killing mechanisms of chimeric antigen receptor (CAR) T cells. Int J Mol Sci. 2019;20:1283.
55. Garg AD, Galluzzi L, Apetoh L, Baert T, Birge RB, Pedro B-S, et al. Molecular and translational classifications of DAMPs in immunogenic cell death. Front Immunol. 2015;6:588.
56. Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-κB pathway. Int Immunol. 2000;12:1539–46.
57. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature. 2002;418:191–5.
58. Corrales L, Matson V, Flood B, Spranger S, Gajewski TF. Innate immune signaling and regulation in cancer immunotherapy. Cell Res. 2017;27:96–108.
59. Kohlhapp FJ, Kaufman HL. Molecular pathways: mechanism of action for talimogene laherparepvec, a new oncolytic virus immunotherapy. Clin Cancer Res. 2016;22:1048–54.
60. Fujiwara T, Urata Y, Tanaka N. Telomerase-specific oncolytic virotherapy for human cancer with the hTERT promoter. Curr Cancer Drug Targets. 2007;7:191–201.
61. Parker BS, Rautela J, Hertzog PJ. Antitumour actions of interferons: implications for cancer therapy. Nat Rev Cancer. 2016;16:131.
62. Dumitriu IE, Baruah P, Valentinis B, Voll RE, Herrmann M, Nawroth PP, et al. Release of high mobility group box 1 by dendritic cells controls T cell activation via the receptor for advanced glycation end products. J Immunol. 2005;174:7506–15.
63. Moser B, Desai DD, Downie MP, Chen Y, Yan SF, Herold K, et al. Receptor for advanced glycation end products expression on T cells contributes to antigen-specific cellular expansion in vivo. J Immunol. 2007;179:8051–8.
64. Haile ST, Conner J, Mackall C. Attenuated oncolytic virus HSV1716 increases in vivo expansion of GD2-targeting CAR T cells in murine solid tumor models. Cancer Res. 2016;76:111–2.
65. Chen X, Han J, Chu J, Zhang L, Zhang J, Chen C, et al. A combinational therapy of EGFR-CAR NK cells and oncolytic herpes simplex virus 1 for breast cancer brain metastases. Oncotarget. 2016;7:27764.
66. Tähtinen S, Grönberg-Vähä-Koskela S, Lumen D, Merisalo-Soikkeli M, Siurala M, Airaksinen AJ, et al. Adenovirus improves the efficacy of adoptive T-cell therapy by recruiting immune cells to and promoting their activity at the tumor. Cancer Immunol Res. 2015;3:915–25.
67. Nakamura K, Smyth MJ. Myeloid immunosuppression and immune checkpoints in the tumor microenvironment. Cell Mol Immunol. 2020;17:1–12.
68. Lindau D, Gielen P, Kroesen M, Wesseling P, Adema GJ. The immunosuppressive tumour network: myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. Immunology. 2013;138:105–15.
69. Whiteside T. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008;27:5904–12.
70. Gurusamy D, Clever D, Eil R, Restifo NP. Novel "elements" of immune suppression within the tumor microenvironment. Cancer Immunol Res. 2017;5:426–33.
71. Azemati F, Kondori BJ, Ghaleh HEG. Therapeutic potential of nanoparticle-loaded hydroxyurea on proliferation of human breast adenocarcinoma cell line. Iran J Pharm Res. 2020;19:271.
72. Achard C, Surendran A, Wedge M-E, Ungerechts G, Bell J, Ilkow CS. Lighting a fire in the tumor microenvironment using oncolytic immunotherapy. EBioMedicine. 2018;31:17–24.
73. Harrington K, Freeman DJ, Kelly B, Harper J, Soria J-C. Optimizing oncolytic virotherapy in cancer treatment. Nat Rev Drug Discov. 2019;18:689–706.
74. Gauvrit A, Brandler S, Sapede-Peroz C, Boisgerault N, Tangy F, Gregoire M. Measles virus induces oncolysis of mesothelioma cells and allows dendritic cells to cross-prime tumor-specific CD8 response. Cancer Res. 2008;68:4882–92.
75. Guillerme J-B, Boisgerault N, Roulois D, Ménager J, Combredet C, Tangy F, et al. Measles virus vaccine–infected tumor cells induce tumor antigen cross-presentation by human plasmacytoid dendritic cells. Clin Cancer Res. 2013;19:1147–58.
76. Moehler MH, Zeidler M, Wilsberg V, Cornelis JJ, Woelfel T, Rommelaere J, et al. Parvovirus H-1-induced tumor cell death enhances human immune response in vitro via increased phagocytosis, maturation, and cross-presentation by dendritic cells. Hum Gene Ther. 2005;16:996–1005.
77. Delaunay T, Violland M, Boisgerault N, Dutoit S, Vignard V, Münz C, et al. Oncolytic viruses sensitize human tumor cells for NY-ESO-1 tumor antigen recognition by CD4+ effector T cells. Oncoimmunology. 2018;7:e1407897.
78. Ilkow CS, Marguerie M, Batenchuk C, Mayer J, Neriah DB, Cousineau S, et al. Reciprocal cellular cross-talk within the tumor microenvironment promotes oncolytic virus activity. Nat Med. 2015;21:530.
79. Li Y, Xiao F, Zhang A, Zhang D, Nie W, Xu T, et al. Oncolytic adenovirus targeting TGF-β enhances anti-tumor responses of mesothelin-targeted chimeric antigen receptor T cell therapy against breast cancer. Cell Immunol. 2020;348:104041.
80. Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RHI, Michielin O, et al. Oncolytic Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. Cell. 2017;170:1109. e10
81. Li Y, Rogoff HA, Keates S, Gao Y, Murikipudi S, Mikule K, et al. Suppression of cancer relapse and metastasis by inhibiting cancer stemness. Proc Natl Acad Sci USA. 2015;112:1839–44.
82. Kottke T, Boisgerault N, Diaz RM, Donnelly O, Rommelfanger-Konkol D, Pulido J, et al. Detecting and targeting tumor relapse by its resistance to innate effectors at early recurrence. Nat Med. 2013;19:1625.
83. Cerullo V, Koski A, V„h„-Koskela M, Hemminki A. Oncolytic adenoviruses for cancer immunotherapy: data from mice, hamsters, and humans. Adv Cancer Res. 2012;115:265–318.
84. Jahan N, Lee JM, Shah K, Wakimoto H. Therapeutic targeting of chemoresistant and recurrent glioblastoma stem cells with a proapoptotic variant of oncolytic herpes simplex virus. Int J Cancer. 2017;141:1671–81.

85. Lang FF, Conrad C, Gomez-Manzano C, Yung WA, Sawaya R, Weinberg JS, et al. Phase I study of DNX-2401 (Delta-24-RGD) oncolytic adenovirus: replication and immunotherapeutic effects in recurrent malignant glioma. J Clin Oncol. 2018;36:1419.
86. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol. 2015;15:486–99.
87. Jin H-T, Anderson AC, Tan WG, West EE, Ha S-J, Araki K, et al. Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. Proc Natl Acad Sci USA. 2010;107:14733–8.
88. Anderson AC. Tim-3: an emerging target in the cancer immunotherapy landscape. Cancer Immunol Res. 2014;2:393–8.
89. Esaki S, Nigim F, Moon E, Luk S, Kiyokawa J, Curry W Jr, et al. Blockade of transforming growth factor-β signaling enhances oncolytic herpes simplex virus efficacy in patient-derived recurrent glioblastoma models. Int J Cancer. 2017;141:2348–58.
90. Hau P, Jachimczak P, Schlaier J, Bogdahn U. TGF-β2 signaling in high-grade gliomas. Curr Pharm Biotechnol. 2011;12:2150–7.
91. Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov. 2015;5:1282–95.
92. Dunn GP, Old LJ, Schreiber RD. The three Es of cancer immunoediting. Annu Rev Immunol. 2004;22:329–60.
93. Fry TJ, Shah NN, Orentas RJ, Stetler-Stevenson M, Yuan CM, Ramakrishna S, et al. CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. Nat Med. 2018;24:20–28.
94. Wing A, Fajardo CA, Posey AD Jr., Shaw C, Da T, Young RM, et al. Improving CART-cell therapy of solid tumors with oncolytic virus-driven production of a bispecific T-cell engager. Cancer Immunol Res. 2018;6:605–16.
95. Berraondo P, Sanmamed MF, Ochoa MC, Etxeberria I, Aznar MA, Pérez-Gracia JL, et al. Cytokines in clinical cancer immunotherapy. Br J Cancer. 2019;120:6–15.
96. Havunen R, Santos JM, Sorsa S, Rantapero T, Lumen D, Siurala M, et al. Abscopal effect in non-injected tumors achieved with cytokine-armed oncolytic adenovirus. Mol Ther Oncolytics. 2018;11:109–21.
97. Durham NM, Mulgrew K, McGlinchey K, Monks NR, Ji H, Herbst R, et al. Oncolytic VSV primes differential responses to immuno-oncology therapy. Mol Ther. 2017;25:1917–32.
98. Cervera-Carrascon V, Siurala M, Santos J, Havunen R, Tähtinen S, Karell P, et al. TNFa and IL-2 armed adenoviruses enable complete responses by anti-PD-1 checkpoint blockade. Oncoimmunology. 2018;7:e1412902.
99. Yang C, Cao H, Liu N, Xu K, Ding M, Mao L-J. Oncolytic adenovirus expressing interleukin-18 improves antitumor activity of dacarbazine for malignant melanoma. Drug Des Devel Ther. 2016;10:3755.
100. Post DE, Sandberg EM, Kyle MM, Devi NS, Brat DJ, Xu Z, et al. Targeted cancer gene therapy using a hypoxia inducible factor–dependent oncolytic adenovirus armed with interleukin-4. Cancer Res. 2007;67:6872–81.
101. Kowalsky SJ, Liu Z, Feist M, Berkey SE, Ma C, Ravindranathan R, et al. Superagonist IL-15-armed oncolytic virus elicits potent antitumor immunity and therapy that are enhanced with PD-1 blockade. Mol Ther. 2018;26:2476–86.
102. Leoni V, Vannini A, Gatta V, Rambaldi J, Sanapo M, Barboni C, et al. A fully-virulent retargeted oncolytic HSV armed with IL-12 elicits local immunity and vaccine therapy towards distant tumors. PLoS Pathog. 2018;14:e1007209.
103. Meisen WH, Kaur B. How can we trick the immune system into overcoming the detrimental effects of oncolytic viral therapy to treat glioblastoma? Expert Rev Neurother. 2013;13:341–3.
104. Choi I, Lee J, Zhang S, Park J, Lee K-M, Sonn C, et al. Oncolytic adenovirus co-expressing IL-12 and IL-18 improves tumor-specific immunity via differentiation of T cells expressing IL-12Rβ 2 or IL-18Rα. Gene Ther. 2011;18:898–909.
105. Bai F-L, Yu Y-H, Tian H, Ren G-P, Wang H, Zhou B, et al. Genetically engineered Newcastle disease virus expressing interleukin-2 and TNF-related apoptosis-inducing ligand for cancer therapy. Cancer Biol Ther. 2014;15:1226–38.
106. Patel MR, Jacobson BA, Ji Y, Drees J, Tang S, Xiong K, et al. Vesicular stomatitis virus expressing interferon-β is oncolytic and promotes antitumor immune responses in a syngeneic murine model of non-small cell lung cancer. Oncotarget. 2015;6:33165.
107. Watanabe K, Luo Y, Da T, Guedan S, Ruella M, Scholler J, et al. Pancreatic cancer therapy with combined mesothelin-redirected chimeric antigen receptor T cells and cytokine-armed oncolytic adenoviruses. JCI Insight. 2018;3:1–17.
108. Hurton LV, Singh H, Najjar AM, Switzer KC, Mi T, Maiti S, et al. Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells. Proc Natl Acad Sci USA. 2016;113:E7788–E7797.
109. Springuel L, Lonez C, Alexandre B, Van Cutsem E, Machiels J-PH, Van Den Eynde M, et al. Chimeric antigen receptor-T cells for targeting solid tumors: current challenges and existing strategies. Biodrugs. 2019;33:515–7.
110. Moon EK, Wang L-CS, Bekdache K, Lynn RC, Lo A, Thorne SH, et al. Intra-tumoral delivery of CXCL11 via a vaccinia virus, but not by modified T cells, enhances the efficacy of adoptive T cell therapy and vaccines. Oncoimmunology. 2018;7:e1395997.
111. Yeku OO, Brentjens RJ, Armored CAR. T-cells: utilizing cytokines and pro-inflammatory ligands to enhance CAR T-cell anti-tumour efficacy. Biochem Soc Trans. 2016;44:412–8.
112. Eckert EC, Nace RA, Tonne JM, Evgin L, Vile RG, Russell SJ. Generation of a tumor-specific chemokine gradient using oncolytic vesicular stomatitis virus encoding CXCL9. Mol Ther Oncolytics. 2020;16:63–74.
113. Liu Z, Ravindranathan R, Li J, Kalinski P, Guo ZS, Bartlett DL. CXCL11-Armed oncolytic poxvirus elicits potent antitumor immunity and shows enhanced therapeutic efficacy. Oncoimmunology. 2016;5:e1091554.
114. Suryadevara CM, Gedeon PC, Sanchez-Perez L, Verla T, Alvarez-Breckenridge C, Choi BD, et al. Are BiTEs the "missing link" in cancer therapy? Oncoimmunology. 2015;4:e1008339.
115. Kantarjian H, Stein A, Gökbuget N, Fielding AK, Schuh AC, Ribera J-M, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. N Engl J Med. 2017;376:836–47.
116. Wang X, Gottschalk S, Song X-T. Synergistic antitumor effects of Chimeric antigen receptor-modified T cells and Oncolytic Virotherapy. Am Soc Hematol. 2014;124:1–21.
117. Dagher O, King TR, Wellhausen N, Posey AD Jr. Combination therapy for solid tumors: taking a classic CAR on new adventures. Cancer Cell. 2020;38:621–3.
118. Scott EM, Duffy MR, Freedman JD, Fisher KD, Seymour LW. Solid tumor immunotherapy with T cell engager-armed oncolytic viruses. Macromol Biosci. 2018;18:1700187.
119. Yu F, Hong B, Song X-T. A T-cell engager-armed oncolytic vaccinia virus to target the tumor stroma. Cancer Transl Med. 2017;3:122.
120. de Sostoa J, Fajardo CA, Moreno R, Ramos MD, Farrera-Sal M, Alemany R. Targeting the tumor stroma with an oncolytic adenovirus secreting a fibroblast activation protein-targeted bispecific T-cell engager. J Immunother Cancer. 2019;7:1–15.
121. Porter CE, Shaw AR, Jung Y, Yip T, Castro PD, Sandulache VC, et al. Oncolytic adenovirus armed with BiTE, cytokine and checkpoint inhibitor enables CAR T-cells to control growth of heterogeneous tumors. Mol Ther. 2020;28:1251–62.
122. Fajardo CA, Guedan S, Rojas LA, Moreno R, Arias-Badia M, de Sostoa J, et al. Oncolytic adenoviral delivery of an EGFR-targeting T-cell engager improves antitumor efficacy. Cancer Res. 2017;77:2052–63.
123. Wing A, Fajardo CA, Posey AD, Shaw C, Da T, Young RM, et al. Improving CART-cell therapy of solid tumors with oncolytic virus–driven production of a bispecific T-cell engager. Cancer Immunol Res. 2018;6:605–16.
124. Zhang S, Rabkin SD. The discovery and development of oncolytic viruses: are they the future of cancer immunotherapy?. Exp Opin Drug Discov. 2020;16:391–410.
125. Le Saux O, Ray-Coquard I, Labidi-Galy SI. Challenges for immunotherapy for the treatment of platinum resistant ovarian cancer. Semin Cancer Biol. 2020;7:135–47.
126. Arcangeli S, Mestermann K, Weber J, Bonini C, Casucci M, Hudecek M. Overcoming key challenges in cancer immunotherapy with engineered T cells. Curr Opin Oncol. 2020;32:398–407.
127. Aalipour A, Le Boeuf F, Tang M, Murty S, Simonetta F, Lozano AX, et al. Viral delivery of CAR targets to solid tumors enables effective cell therapy. Mol. Ther Oncolytics. 2020;17:232–40.
128. Tang X, Li Y, Ma J, Wang X, Zhao W, Hossain MA, et al. Adenovirus-mediated specific tumor tagging facilitates CAR-T therapy against antigen mismatched solid tumors. Cancer Lett. 2020;487:1–19.
129. Park AK, Fong Y, Kim S-I, Yang J, Murad JP, Lu J, et al. Effective combination immunotherapy using oncolytic viruses to deliver CAR targets to solid tumors. Sci Transl Med. 2020;12:1–13.
130. Tagliamonte M, Mauriello A, Cavalluzzo B, Ragone C, Manolio C, Petrizzo A, et al. Tackling hepatocellular carcinoma with individual or combinatorial immunotherapy approaches. Cancer Lett. 2020;473:25–32.
131. Hai J, Zhang H, Zhou J, Wu Z, Chen T, Papadopoulos E, et al. Generation of genetically engineered mouse lung organoid models for squamous cell lung cancers allows for the study of combinatorial immunotherapy. Clin Cancer Res. 2020;26:3431–42.
132. Garcia-Carbonero R, Salazar R, Duran I, Osman-Garcia I, Paz-Ares L, Bozada JM, et al. Phase 1 study of intravenous administration of the chimeric adenovirus enadenotucirev in patients undergoing primary tumor resection. J Immunother Cancer. 2017;5:71.
133. Heinzerling L, Künzi V, Oberholzer PA, Kündig T, Naim H, Dummer R. Oncolytic measles virus in cutaneous T-cell lymphomas mounts antitumor immune responses in vivo and targets interferon-resistant tumor cells. Blood. 2005;106:2287–94.

134. Turtle CJ, Hanafi L-A, Berger C, Gooley TA, Cherian S, Hudecek M, et al. CD19 CAR–T cells of defined CD4+: CD8+ composition in adult B cell ALL patients. J Clin Invest. 2016;126:2123–38.
135. Peng K-W, Myers R, Greenslade A, Mader E, Greiner S, Federspiel MJ, et al. Using clinically approved cyclophosphamide regimens to control the humoral immune response to oncolytic viruses. Gene Ther. 2013;20:255–61.
136. Gattinoni L, Finkelstein SE, Klebanoff CA, Antony PA, Palmer DC, Spiess PJ, et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J Exp Med. 2005;202:907–12.
137. Esmaeili Govarchin Ghaleh H, Zarei L, Mansori Motlagh B, Jabbari N. Using CuO nanoparticles and hyperthermia in radiotherapy of MCF-7 cell line: synergistic effect in cancer therapy. Artif Cells Nanomed Biotechnol. 2019;47:1396–403.
138. Akram A, Inman RD. Immunodominance: a pivotal principle in host response to viral infections. Clin Immunol. 2012;143:99–115.
139. Frahm N, DeCamp AC, Friedrich DP, Carter DK, Defawe OD, Kublin JG, et al. Human adenovirus-specific T cells modulate HIV-specific T cell responses to an Ad5-vectored HIV-1 vaccine. J Clin Invest. 2012;122:359–67.
140. Galivo F, Diaz RM, Thanarajasingam U, Jevremovic D, Wongthida P, Thompson J, et al. Interference of CD40L-mediated tumor immunotherapy by oncolytic vesicular stomatitis virus. Hum Gene Ther. 2010;21:439–50.
141. Rodriguez-Garcia A, Svensson E, Gil-Hoyos R, Fajardo C, Rojas L, Arias-Badia M, et al. Insertion of exogenous epitopes in the E3-19K of oncolytic adenoviruses to enhance TAP-independent presentation and immunogenicity. Gene Ther. 2015;22:596–601.
142. Pule MA, Savoldo B, Myers GD, Rossig C, Russell HV, Dotti G, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nat Med. 2008;14:1264–70.
143. Melzer MK, Zeitlinger L, Mall S, Steiger K, Schmid RM, Ebert O, et al. Enhanced safety and efficacy of oncolytic VSV therapy by combination with T cell receptor transgenic T cells as carriers. Mol Ther Oncolytics. 2019;12:26–40.
144. Todo T, Tanaka M, Ito M, Ito H, Ino Y. Clinical trials of a third-generation recombinant oncolytic HSV-1 in recurrent glioblastoma and olfactory neuroblastoma patients. Neuro Oncol. 2014;16:iii51–iii51.
145. Markert JM, Liechty PG, Wang W, Gaston S, Braz E, Karrasch M, et al. Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM. Mol Ther. 2009;17:199–207.
146. Breitbach CJ, Moon A, Burke J, Hwang TH, Kirn DH. A Phase 2, open-label, randomized study of Pexa-Vec (JX-594) Administered by intratumoral injection in patients with unresectable primary hepatocellular carcinoma. Methods Mol Biol. 2015;1317:343–57.
147. Packiam VT, Lamm DL, Barocas DA, Trainer A, Fand B, Davis RL 3rd, et al. An open label, single-arm, phase II multicenter study of the safety and efficacy of CG0070 oncolytic vector regimen in patients with BCG-unresponsive non-muscle-invasive bladder cancer: interim results. Urol Oncol. 2018;36:440–7.
148. Black AJ, Morris DG. Clinical trials involving the oncolytic virus, reovirus: ready for prime time? Expert Rev Clin Pharmacol. 2012;5:517–20.
149. Jaime-Ramirez AC, Yu JG, Caserta E, Yoo JY, Zhang J, Lee TJ, et al. Reolysin and histone deacetylase inhibition in the treatment of head and neck squamous cell carcinoma. Mol Ther Oncolytics. 2017;5:87–96.
150. Lauer U, Zimmermann M, Sturm J, Koppenhoefer U, Bitzer M, Malek NP, et al. Phase I/II clinical trial of a genetically modified and oncolytic vaccinia virus GL-ONC1 in patients with unresactable, chemotherapy-resistant peritoneal carcinomatosis. Am Soc Clin Oncol. 2013;31:127–40.
151. Andtbacka RHI, Curti BD, Kaufman H, Daniels GA, Nemunaitis JJ, Spitler LE, et al. Final data from CALM: A phase II study of Coxsackievirus A21(CVA21) oncolytic virus immunotherapy in patients with advanced melanoma. Am Soc Clin Oncol. 2015;33:101–10.
152. Merchan JR, Patel M, Cripe TP, Old MO, Strauss JF, Thomassen A, et al. Relationship of infusion duration to safety, efficacy, and pharmacodynamics(PD): Second part of a phase I-II study using VSV-IFNβ-NIS (VV1) oncolytic virus in patients with refractory solid tumors. Am Soc Clin Oncol. 2020;38:212–9.
153. Fueyo J, Gomez-Manzano C, Villalobos P, Rodriguez-Canales J, Wistuba I, Hess KR, et al. ATIM-08. immunomarkers in the DNX-2401 (DELTA-24-RGD) oncolytic virus phase I clinical trial. Neuro Oncol. 2017;19:vi27–vi27.
154. Desjardins A, Gromeier M, Herndon JE 2nd, Beaubier N, Bolognesi DP, Friedman AH, et al. Recurrent glioblastoma treated with recombinant poliovirus. N Engl J Med. 2018;379:150–61.
155. Foloppe J, Kempf J, Futin N, Kintz J, Cordier P, Pichon C, et al. The enhanced tumor specificity of TG6002, an armed oncolytic vaccinia virus deleted in two genes involved in nucleotide metabolism. Mol Ther Oncolytics. 2019;14:1–14.
156. Dispenzieri A, Tong C, LaPlant B, Lacy MQ, Laumann K, Dingli D, et al. Phase I trial of systemic administration of Edmonston strain of measles virus genetically engineered to express the sodium iodide symporter in patients with recurrent or refractory multiple myeloma. Leukemia. 2017;31:2791–8.
157. Louis CU, Savoldo B, Dotti G, Pule M, Yvon E, Myers GD, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood. 2011;118:6050–6.
158. Katz SC, Burga RA, McCormack E, Wang LJ, Mooring W, Point GR, 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;21:3149–59.
159. Zhang C, Wang Z, Yang Z, Wang M, Li S, Li Y, et al. Phase I escalating-dose trial of CAR-T therapy targeting CEA(+) metastatic colorectal cancers. Mol Ther. 2017;25:1248–58.
160. Feng K, Guo Y, Dai H, Wang Y, Li X, Jia H, et al. Chimeric antigen receptor-modified T cells for the immunotherapy of patients with EGFR-expressing advanced relapsed/refractory non-small cell lung cancer. Sci China life Sci. 2016;59:468–79.
161. Ahmed N, Brawley V, Hegde M, Bielamowicz K, Wakefield A, Ghazi A, et al. Autologous HER2 CMV bispecific CAR T cells are safe and demonstrate clinical benefit for glioblastoma in a Phase I trial. J Immunother Cancer. 2015;3:1–1.
162. Feng K, Liu Y, Guo Y, Qiu J, Wu Z, Dai H, et al. Phase I study of chimeric antigen receptor modified T cells in treating HER2-positive advanced biliary tract cancers and pancreatic cancers. Protein Cell. 2018;9:838–47.
163. O'Rourke DM, Nasrallah M, Morrissette JJ, Melenhorst JJ, Lacey SF, Mansfield K. et al. Pilot study of T cells redirected to EGFRvIII with a chimericantigen receptor in patients with EGFRvIII+ glioblastoma. Am Soc Clin Oncol. 2016;34:183–92.
164. Katz SC, Hardaway J, Prince E, Guha P, Cunetta M, Moody A, et al. HITM-SIR: phase Ib trial of intraarterial chimeric antigen receptor T-cell therapy and selective internal radiation therapy for CEA+ liver metastases. Cancer Gene Ther. 2020;27:341–55.
165. Nishio N, Diaconu I, Liu H, Cerullo V, Caruana I, Hoyos V, et al. Armed oncolytic virus enhances immune functions of chimeric antigen receptor–modified T cells in solid tumors. Cancer Res. 2014;74:5195–205.
166. Shaw AR, Porter CE, Watanabe N, Tanoue K, Sikora A, Gottschalk S, et al. Adenovirotherapy delivering cytokine and checkpoint inhibitor augments CAR T cells against metastatic head and neck cancer. Mol Ther. 2017;25:2440–51.
167. Tanoue K, Shaw AR, Watanabe N, Porter C, Rana B, Gottschalk S, et al. Armed oncolytic adenovirus–expressing PD-L1 mini-body enhances antitumor effects of chimeric antigen receptor T cells in solid tumors. Cancer Res. 2017;77:2040–51.

AUTHOR CONTRIBUTIONS

All authors equally contribute to this study.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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