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REVIEW ARTICLE

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Improvement of current immunotherapies with engineered oncolytic viruses that target cancer stem cells

Alborz Soroush[1] | Reza Shahhosseini[2] | Nima Ghavamikia[3] | Ahmed Hjazi[4] | Shahrzad Roudaki[5] | Mahdi KhalatbariLimaki[6] | Mahtab Mirbolouk[7] | SeyedAbbas Pakmehr[8] | Parvin Karimi[9]

[1]Ramsay Pharmacy Group, Melbourne, Australia

[2]Faculty of Medicine, Istanbul Medipol University, Istanbul, Turkey

[3]Cardiovascular Research Institute, Tehran Heart Center, Tehran University of Medical Sciences, Tehran, Iran

[4]Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Sattam bin AbdulAziz University, Al-Kharj, Saudi Arabia

[5]School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

[6]School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran

[7]School of Pharmacy, Cyprus International University, Nicosia, North Cyprus

[8]School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

[9]Fars Population-Based Cancer Registry, Shiraz University of Medical Sciences, Shiraz, Iran

Correspondence
SeyedAbbas Pakmehr
Email: pakmehrabbas@yahoo.com

Parvin Karimi
Email: parvinkarimi@sums.ac.ir

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Abstract

The heterogeneity of the solid tumor microenvironment (TME) impairs the therapeutic efficacy of standard therapies and also reduces the infiltration of antitumor immune cells, all of which lead to tumor progression and invasion. In addition, self-renewing cancer stem cells (CSCs) support tumor dormancy, drug resistance, and recurrence, all of which might pose challenges to the eradication of malignant tumor masses with current therapies. Natural forms of oncolytic viruses (OVs) or engineered OVs are known for their potential to directly target and kill tumor cells or indirectly eradicate tumor cells by involving antitumor immune responses, including enhancement of infiltrating antitumor immune cells, induction of immunogenic cell death, and reprogramming of cold TME to an immune-sensitive hot state. More importantly, OVs can target stemness factors that promote tumor progression, which subsequently enhances the efficacy of immunotherapies targeting solid tumors, particularly the CSC subpopulation. Herein, we describe the role of CSCs in tumor heterogeneity and resistance and then highlight the potential and remaining challenges of immunotherapies targeting CSCs. We then review the potential of OVs to improve tumor immunogenicity and target CSCs and finally summarize the challenges within the therapeutic application of OVs in preclinical and clinical trials.

KEYWORDS
cancer stemness, drug resistance, immunotherapy, oncolytic viruses, tumor microenvironment

1 | INTRODUCTION

Malignant tumors of the human body with a poor prognosis and a low survival rate cause an increasing number of deaths annually throughout the world.[1] Despite current standard therapies (i.e., chemotherapy, radiotherapy [RT], immunotherapy, and combination therapies) and the application of new diagnostic or detection methods, the maximum survival rate, reaching an average of 20 months, and is affected by high recurrence rates in glioblastoma (GBM).[2,3] Although immune checkpoint blockade (ICB) and CAR T-cell-based immunotherapies show promise in some cancers, they have shown limited efficacy in solid tumors with high dormancy and recurrence rates.[4,5]

Genetic and epigenetic heterogeneity of tumors, a major hallmark of advanced solid cancers, along with cold and complicated tumor microenvironment (TME) inducing the immunosuppressive

niche, are other major reasons that intensify therapy resistance in human tumors. In addition, factor-driven stemness and tumor-associated cancer stem cells (CSCs) are the leading players in this framework that contribute to tumorigenesis, progression, recurrence, metastasis and resistance to current treatment, and even intertumoral cellular heterogeneity.[6,7] Therefore, it is important to gain a comprehensive understanding of the resistance mechanisms and then develop targeted therapies against the stemness-supportive factors or CSCs to increase the efficacy of targeted therapy and improve the persistent poor disease outcome.

Oncolytic viruses (OVs) are types of viruses that selectively infect and lyse malignant cells, subsequently releasing pathogen-associated molecular patterns and damage-associated molecular patterns (DAMPs) to prepare tumor niches for antitumor immune responses.[8] In addition, local or systemic administration of OVs has increased the infiltration of effector lymphocytes into tumor sites while decreasing the levels of tumor-supportive immune cells such as regulatory T cells (Tregs), tumor-associated macrophages, and myeloid-derived suppressor cells (MDSCs).[9] Thereby, leveraging manipulated or engineered OVs has emerged as a promising therapeutic strategy that could target CSCs or stemness and reshape TME to overcome immunotherapies resistance, as presented in this review.

Significance statement

- Oncolytic viruses (OVs) can target cancer stem cells (CSCs) and reprogram the tumor microenvironment by recruiting immune cells to tumor sites.
- Targeting CSCs with engineered OVs improves the treatment of resistant tumors.
- The combination of OVs has improved the therapeutic outcomes of current immunotherapies.

2 | STEMNESS FACTORS AND IMMUNOTHERAPY RESISTANCE

The capacity of self-renewal and differentiation into multiple cellular subtypes introduces CSCs as the source or origin of both tumor initiation and tumor maintenance in various cancers. TME-associated stromal and non-stromal cells more support tumorigenic function of CSCs through activation of survival-supportive signaling pathways such as Wnt/β-catenin, Hh, Notch, and NF-κB and on the other hand CSCs-derived immunomodulators (i.e., cytokines and chemokines), angiogenic factors (e.g., VEGF, vascular endothelial growth factor), and metastatic factors (e.g., MMP-2, matrix metalloproteinase-2) lead to recruitment of tumor-supportive immune cells, activation of angiogenesis, and promotion of tumor metastasis.[10,11] Various mechanisms of radio/chemoresistance in CSCs have emerged, resulting in the development of specific approaches for CSC-directed immunotherapies including: (i) monoclonal and bispecific antibodies targeting CSCs surface biomarkers (e.g., CD44v6, CD44, CD123, EGFR, EpCAM, and CD133) or immune checkpoint molecules using immune checkpoint inhibitors (ICIs) (e.g., Letaplimab against CD47), (ii) adoptive cell therapy in the format of chimeric antigen receptor (CAR) T-cells and CAR NK-cells, and (iii) CSCs-based vaccines[12] (Figure 1 and Table 1).

2.1 | Therapeutic antibodies targeting CSCs

Therapeutic antibodies activate immune cells against tumor-associated antigen (TAA)-expressing CSCs or engage effector immune T cells and NK cells through antigen delivery to dendritic cells (DCs) as professional antigen-presenting cells (APCs). They also target immune checkpoint molecules such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and its ligand, PD-L1, to restore immune cell function. In addition, therapeutic antibodies induce antibody-dependent cellular cytotoxicity by NK cells to activate cell-mediated cytotoxicity (Figure 1A and Table 1). However, monoclonal antibodies (mAb) targeting TAA resulted in fatal side effects due to sharing antigen with normal cells of human body [28] and although developed recombinant humanized antibodies have shown acceptable safety profile, however their clinical efficacy was limited to liquid tumors such as acute myeloid leukemia.[29] The bi- and multi-specific antibodies (BsAb and MsAb) (e.g., CD123 × CD3 BsAb and CD47 × PD-1 BsAb) have been developed to simultaneously target effector immune cells and tumor cells or CSCs and have even been used in clinical trials for relapsed/refractory hematological malignancies (NCT02152956 and NCT0409776), where they were well-tolerated and showed potent antitumor activity. However, similar to mono-specific antibodies, these types of therapeutic antibodies failed to treat solid tumors. Notably, in terms of tumor stemness c-Met expression, a c-Met × CTLA-4 BsAb targeting c-Met in CSCs and CTLA-4 in Tregs showed significant antitumor activity in lung cancer models,[30] and Amivantamab, an EGFR/c-Met specific-BsAb has been clinically approved for the treatment of patients with NSCLC.[31] Overall, loss of the tumor antigen, off-target effects, poor penetration into solid tumors, CSC heterogeneity and plasticity, and poor accessibility to CSCs in hypoxic core of tumor mass limited therapeutic efficacy of antibodies.[32,33]

2.2 | Immune cells targeting CSCs

The potentials of tumor-associated effector T cells and NK cells to induce tumor cytolysis has led to the development and manipulation of these cells toward ex vivo expansion of conventional TCRs that recognize the MHC molecules bearing TAA on APCs or production of genetically engineered CAR T/NK cells that directly target TAA on tumor cells in an MHC-independent manner[34] (Figure 1B). In addition to the development of CAR T-cells targeting TAA (e.g., HER2, EGFR, EpCAM, etc.), several clinical trials have been conducted for CAR T-cell-based therapies targeting CSC markers (e.g., CD44v6, CD133,

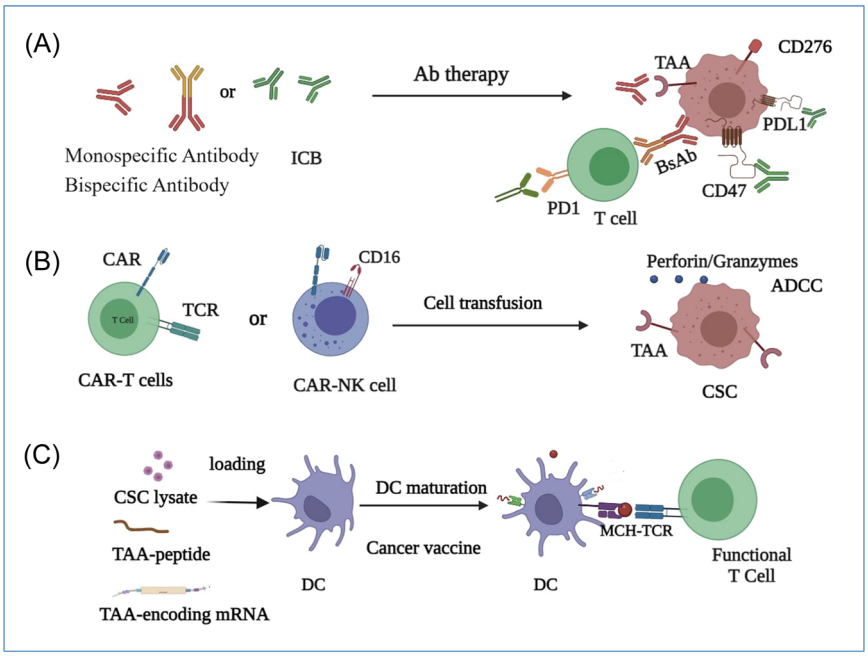

FIGURE 1 Developed immunotherapies for targeting cancer stem cells (CSCs). (A) Systemic administration of monospecific antibodies or bispecific antibodies and immune checkpoint blockade (ICB) leads to targeting of CSC-specific antigens or immune checkpoint molecules (PD1, PDL1, CD276, and CD47) on CSCs/T cells, resulting in induction of antitumor response or recovering exhausted T cells. (B) Transfused CAR-T cells or CAR-NK cells target tumor-associated antigen (TAA) on CSCs and may induce cytolysis and apoptosis. In addition, antibodies targeting TAA on CSCs and CD16 on NK cells mediate the antibody-dependent cellular cytotoxicity (ADCC) process. (C) As an anticancer vaccine, DCs loaded with CSC lysates or TTA peptide or mRNA encoding TAA that, after administration, activate or boost cytotoxic T cells in an MHC-TCR-dependent manner to eradicate CSCs. CSCs, cancer stem cells; DCs, dendritic cells.

EpCAM, MUC1, and TM4SF1) (Table 1). The Mucin 1 (MUC1) was characterized as stemness driver thou upregulation of CSC markers such as ALDH1, BMI1, Oct4, Nanog, Sox2, and LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5) and CAR NK-call targeting is in Phase I/II of clinical trials (NCT02839954).[35,36] About five CAR-T-based products have been approved for the treatment of hematological malignancies. However, their clinical application in solid tumors is limited due to several challenges such as in vitro cost, in vivo off-target toxicity and CAR T-cell exhaustion, immunosuppressive TME with poor CAR T-cell tumor infiltration.[37]

Thereafter, new generations of CAR T-cells (i.e., bispecific CARs, multi-CARs, adapter CAR T-cells) were developed to reduce unwanted off-tumor effects of the CAR T-cell product against healthy tissues.[12] Compared to CAR T cells, CAR NK cell therapy induces less CRS, has a low risk of graft-versus-host disease, and is cost-effective because it can be prepared from multiple sources[38] (Table 1).

However, their clinical application is challenged by the short in vivo lifespan and limited proliferative capacity as well as durable therapeutic responses.[39] Tumor heterogeneity, immunosuppressive TME, off-tumor cell killing, and poor CAR NK infiltration into solid tumors also reduce the CAR NK anticancer efficacy.[12,40] Overall, the same challenges remain with CAR immune cells that need to be addressed in ongoing and future studies.

2.3 | CSC-based DC vaccine

DC-based vaccines are most commonly produced by ex vivo differentiation of autologous progenitor cells into immature DCs, maturation of the DCs by the addition of a cytokine cocktail, and subsequent pulsing with cancer antigens in the form of antigenic peptides, tumor cell lysates, exosomes, or mRNA. The mature DCs are then reinfused into the patient, where they activate antigen-specific T cells.[41] CSC-derived antigens could be processed by DC, and then their epitopes presented by expressing MHC II and MHC I molecules to CD4 + T cells and CD8 + T cells, respectively, followed by effector T cell activation and tumor eradication. Therefore, ex vivo exposure of DC to CSC antigenic peptides or mRNAs and CSC lysates and readministering to patients to elicit T cell responses is a promising strategy for developing CSC-based vaccines (Figure 1C and Table 1). Intravenous (IV) injection of ALDH peptide-loaded DC into immuno-deficient mice resulted in activation of Ag-specific CD8 + T cells and inhibition of primary tumors in xenografted lung tumors.[42] ALDH is a family of metabolic enzymes responsible for detoxifying intracellular aldehydes by oxidizing them to carboxylic acids. High ALDH activity, measured by ALDEFLUOR analysis, is used as a marker to isolate CSC populations in many solid malignancies. DCs pulsed with ALDH1 peptide and injected intravenously into the immunocompromised xenografted mice showed that ALDH1 peptide-specific CD8 + T cells inhibited subcutaneous primary tumor growth and lung metastases.[42]

TABLE 1 Immunotherapeutic methods for targeting CSCs.

Type of immunotherapy	Target	Cancer type	Status	Study results	Reference
Monoclonal antibodies/immune checkpoint inhibitors					
Checkpoint inhibitors	PDL-1	HCC	Phase III	Meaningful survival and a safety profile	NCT03434379
mAb	CD44	Malignant solid tumors	Phase I	Well-tolerated and clinical efficacy were modes	NCT01358903
mAb	CD8	Metastatic cancers	Phase II	Increased tumoral infiltration of CD8 + T cells on treatment	NCT03651271
Fusion of anti-CD123 ScFv and anti-CD3 ScFv	CD123/CD3	AML CSCs	In vitro	Achieve T-cell-mediated target cell killing activities at low pM levels	[13]
OMP-52M51	Notch 1	Breast cancer CSCs	In vivo	Specifically recognizes the Notch1 intracellular domain	[14]
Demcizumab	DLL4	Metastatic NSCLC	Phase IB	50% of patients had objective tumor responses	[15]
Solitomab (MT110)	EpCAM	Pancreatic and colon CSCs	In vitro and in vivo	MT110 prevented the growth of tumors from a 5000-fold	[16, 17]
CAR-T-cell therapy					
AC133-CAR T cells	AC133 epitope of CD133	CD57+ patient-derived GBM-SCs	In vitro and in vivo	CD57 was upregulated on activated T cells only upon contact with CD57+ patient-derived GBM-SCs,	[18]
PD-1-deficient CD133-specific CAR T cells	CD133	CD133-OE luc cells	In vivo	Enhanced inhibition of tumor growth in vivo	[19]
Autologous CD44v6 CAR T-cells	CD44v6	AML and multiple myeloma	Phase I–IIa	D44v6-NWN2.CAR T cells are enriched in central memory cells and show improved in vivo functions	NCT04097301
Anti-GD2 CAR-T-cells	CSPG4	Glioblastoma CSCs	In vitro	Anti-CSPG4 CAR-transduced T cells recognize and kill these GSC	[20]
CAR-NK-cell therapy					
CD33-CAR-NK-92-cells	CD33	AML	Phase 1/2	No significant adverse effects were observed	NCT02944162
CAR-NK92 cells	CD133	Ovarian cancer	In vivo	Showed specific killing against CD133-positive ovarian cancer cells	[21]
CAR-NK-cells	CD24 and mesothelin	Ovarian CSCs and non-stem cell tumor cells	In vivo	Showed a highly cytotoxic effect against OC	[22]
CAR-NK92 cells	EpCAM	Colorectal CSCs		Specifically, recognize EpCAM-positive colorectal cancer cells and release cytokines	[23]
CSC-based vaccines					
CSC lysate-loaded DCs	CSCs	Melanoma	In vivo	Produce specific cytotoxic responses to CSCs	[24]
GSC-pulsed dendritic cell vaccine	Glioblastoma stem cell-like (GSC)	Glioblastoma	GBM patients	Prolonged OS and PFS and increased plasma levels of cytokines CCL22 and IFN-γ	[25] NCT 01567202

TABLE 1 (Continued)

Type of immunotherapy	Target	Cancer type	Status	Study results	Reference
DCs loaded with 9L CSCs	9L CSC tumors	CSC brain tumor model	In vivo	induced CTLs against CSCs, and prolonged survival in animals' model	[26]
DCs pulsed with CSC total RNA	CSCs	Breast cancer	In vitro	CSC RNA was a more efficient antigen source compared with RNA from mixed BC cells and the CSC-specific effector T cells significantly induced BC cell apoptosis	[27]

Abbreviations: AML, acute myeloid leukemia; CSCs, cancer stem cells; DCs, dendritic cells; mAb, monoclonal antibodies.

Clinical trials studies using MUC1-derived peptide that were well-tolerated and resulted in promising clinical responses in patients with refractory NSCLC, pancreatic cancer, biliary cancer, and chemoresistant prostate cancer.[43]

However, immunotherapy using peptide-based DC vaccines against a single antigen is limited by the heterogeneity and plasticity of antigen expression in CSCs. Therefore, pulsing DCs with the entire CSC lysate, potentially including the entire repertoire of tumor antigens, is another promising strategy for the development of CSC-specific DC vaccines.[44] More efficient than peptide-based DC vaccines is the loading of DC with CSC lysates or CSC-isolated mRNA encoding multiple antigens can induce rebuts cytotoxic T cell responses.[27,45] Antitumor immunity induced by vaccination with murine bone marrow DCs pulsed with $ALDH^{high}$ CSC tumor lysate could induce significantly higher protective immunity against DCs pulsed with ALDH-negative cell lysate.[46] Antitumor immunogenicity could also be enhanced by targeting multiple epitopes and pulsing DCs with CSC-derived mRNA. In a preclinical study, PBMCs-DCs were pulsed with mRNA isolated from the CD44+/CD24− CSC population. The results showed that CSC mRNA induced a more potent cytotoxic T-cell response compared to mRNA isolated from the whole tumor cell population.[27]

However, monotherapy with DC-based vaccines alone does not induce sufficient outcomes for cancer treatment and demands combination with other therapies such as ICB and tumor RT, and chemotherapy (Table 2), and thus, these vaccines might act as adjuvant to optimize their therapeutic efficacy. $ALDH^{high}$ CSC lysate pulsed DCs combined with local tumor RT demonstrated that it has higher anticancer efficacy in the adjuvant setting when administered after RT.[57] The combination of cisplatin-based chemotherapy with a DC vaccine targeting the CD44+/CD24− CSC-like cell population may improve the efficacy of cisplatin-based chemotherapy for the treatment of murine Ehrlich tumors.[50] In conclusion, immune-targeting of CSCs is a promising approach for cancer treatment. In addition, a strategy to optimize their therapeutic efficacy may be to combine them with ICIs and conventional therapies such as surgery, chemotherapy, and RT.

3 | ONCOLYTIC VIRUS IMPROVES CSC-CASED IMMUNOTHERAPY

3.1 | OVs induce immunogenic TME

Aside from direct lysis of cancer cells, OVs are known to improve tumor immunotherapy through two major mechanisms including induction of proinflammatory molecules to create hot TME and enhancing tumor infiltration, particularly APCs, CD4+, and CD8 + T cells and also their maturation and activation toward providing immunogenic TME. Immunosuppressive TME also limits the results of ICB therapy due to the induction of T-cell exhaustion, and therefore, combination therapy such as OV therapy could be an innovative approach to increase the benefits of ICB therapy. For example, ZIKV

TABLE 2 Combination therapies targeting CSC markers.

Immunotherapy	Target	Combined with	Cancer type	Status	Study results	References
Magrolimab (Hu5F9-G4)	CD47 on leukemia stem cells (LSC)	Azacitidine (AZA)	AML/MDS	NCT03248479	Well-tolerated with robust activity in MDS and AML patients with an ORR of 100% and 69%, respectively	[47]
Cirmtuzumab	ROR1 (onco-embryonic tyrosine kinase receptor)	Ibrutinib	CLL	NCT03088878	High ORR and PFS are encouraging, particularly for relapsed CLL	[48]
Demcizumab (OMP-21M18)	Delta-like ligand 4 (DLL4)	Carboplatin + pemetrexed	Non-squamous NSCLC	NCT02259582	64% of evaluable patients at 10 mg/kg had evidence of stabilization of disease or response	[49]
CD44+/CD24−CSC-pulsed DC vaccine	CD44+/CD24− CSCs	Cisplatin (DNA-binding cytotoxic drug)	Ehrlich carcinoma	In vivo (cell-line derived xenograft)	CSC-pulsed DCs significantly downregulated ($p < .001$) Bcl-2 gene expression	[50]
Anti-CD133 CAR-T cells	CD133	Cisplatin	Gastric cancer	In vivo (cell-line derived xenograft)	Inhibited tumor progression in xenograft models with diminished CD133 positive stem cell-like cell infiltration	[51]
ALDH ^{High}-DC vaccine	ALDH ^{High} CSCs	Anti-CTLA-4 antibody and anti-PD-L1 antibody	B16-F10 murine melanoma tumors	In vivo (syngeneic models)	Resulted in ~1.7-fold fewer PD-1 + CD8 + T cells and ~2.5-fold fewer CTLA-4 + CD8 + T cells	[52]
Tocilizumab	IL-6	MK-0752 (γ-secretase inhibitor)	Breast cancer	In vivo (patient-derived xenograft)	Significantly decreases BCSCs and inhibits tumor growth	[53]
HER2 CAR NK-92	HER2	Apatinib (VEGFR-2 inhibitor)	Gastric cancer	In vivo (cell-line derived xenograft)	Increased NK cell infiltration and improved the therapeutic efficacy of NK-92 cells	[54]
EGFRCAR NK-92	EGFR	Cabozantinib (VEGFR-2 inhibitor)	Renal cell carcinoma	In vivo (cell-line derived xenograft)	Show synergistic therapeutic efficacy with Cabozantinib against human RCC xenograft models	[55]
CAR T cell	Carbonic anhydrase IX (CAIX)	Sunitinib (multitargeted receptor kinase inhibitor)	Renal cell carcinoma	In vivo (cell-line derived xenograft)	Releasing anti-PD-L1 IgG highly decreases both tumor volume and weight in vivo, avoiding the occurrence of metastasis	[56]

Abbreviations: AML, acute myeloid leukemia; CSCs, cancer stem cells; DCs, dendritic cells; VEGF, vascular endothelial growth factor.

TABLE 3 Oncolytic virus in combination therapy targeting if cancer stem cells.

Oncolytic virus	Combined with	Types of CSCs	In vitro/In vivo study	Major finding	References
oHSV G47D	MG18L (TGF-β inhibitors)	Glioblastoma-derived stem cell from	Orthotopic recurrent GSC xenograft SCID mice model	Synergistic in killing recurrent GSCs	[60]
G47DΔmIL1	VEGFR Inhibitor Axitinib	patient-derived recurrent MGG123 GSCs	Patient/mouse-derived recurrent GSC xenografts in immunodeficient mice	Enhance antitumor efficacy in both GBM models	[61]
Parvovirus H-1 (H-1PV)	valproic acid	GBM "stem-like" cells. cervical and pancreatic carcinomas	High-grade glioma stem-like cells in NOD/SCID mice/carcinoma cell lines	H-1PV induces complete cell death in all neurosphere cultures. H-1PV/VPA cotreatment strongly inhibits tumor growth	[62, 63]
oHSV G47D expressing murine IL-12 (G47D-mIL12)	Anti-CTLA-4, anti-PD-1	Glioblastoma stem-like cells (GSCs)	Syngeneic orthotopic mouse model	M1-like polarization, along with increased T effector to T regulatory cell ratios	[64]
mGM-CSF carrying oncolytic adenovirus (OAV)	11R-P53 and SG655-mGMP	Hepatocellular carcinoma stem cells (Hep3B-C)	Teratoma stem cells (ECCG5)/ECCG5 stem cell-formed C57BL/6 mouse xenografts model	Induced significant xenograft growth inhibition and immune cell infiltration	[65]
CD133-targeted oncolytic measles virus (MV-CD133)	Vesicular stomatitis virus	Hepatocellular CSC	Hepatocellular cancer mice model	Highly selective in eliminating CD133-positive cells	[66]
Recombinant vesicular stomatitis virus (VSV-ΔM51)	double deleted Vaccinia Virus (vvDD)	GBM	animal glioma models.	VSV-ΔM51and vvDD could infect and kill TMZ-resistant brain tumor stem cells	[67]
Measles viruses (MV)	DARPins	Human ovarian adenocarcinoma stem cell	Ovarian cancer xenograft model in mice	Simultaneously target tumor marker and HER2/neu CSC-marker EpCAM	[68]
Cancer-favoring oncolytic vaccinia virus (CVV)	Drug 5-Fu	Stem cell-like colon cancer cells (SCCs)	Syngeneic carcinomatosis mouse model	Synergistically enhanced by simultaneous treatment	[69]
Oncolytic measles virus (MeV)	Radiotherapy, or TMZ) or lomustine	Glioma stem-like	Glioma cell lines LN229, LNZ308, and glioma stem-like GS8 cells	Displayed synergistic anti-glioma activity	[70]
oHSV	TRAIL	GBM stem cells (GSC)	Tumor-bearing mice	Overcome the therapeutic resistance and cures in 40% of the treated mice	[71]

Abbreviations: CSCs, cancer stem cells; VEGF, vascular endothelial growth factor.

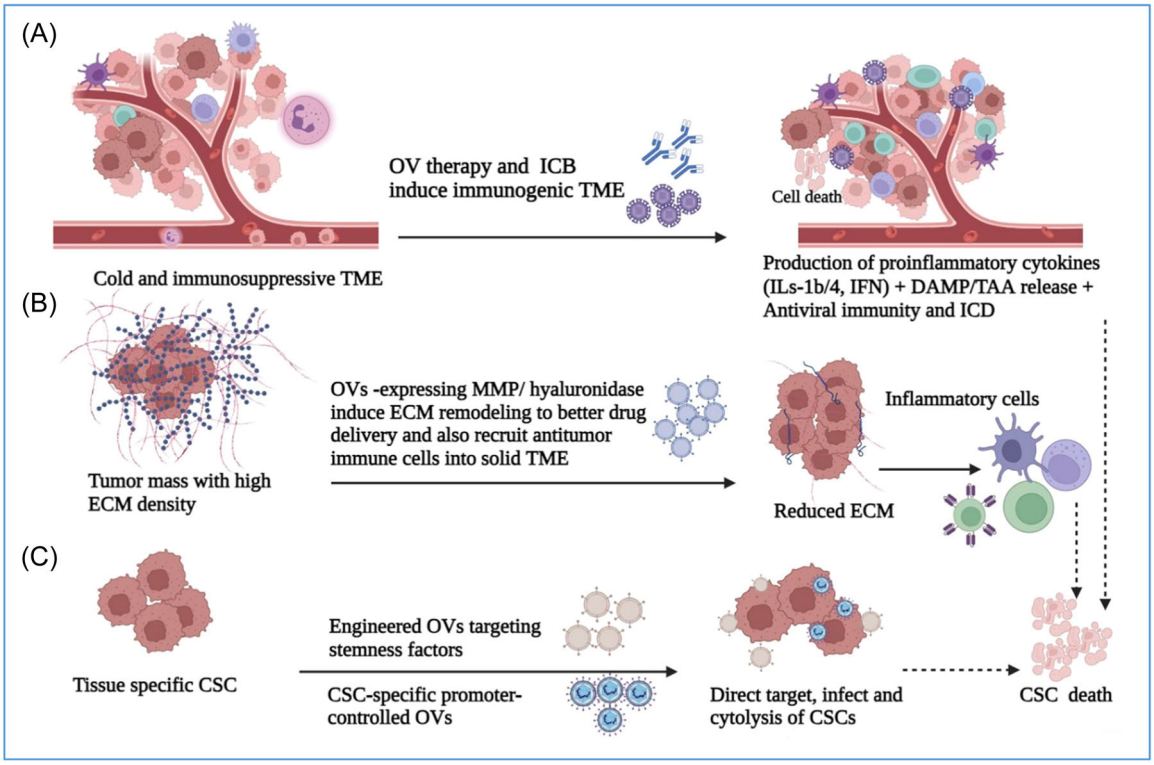

FIGURE 2 OV therapy improves CSC-immunotherapy through (A) induction of immunogenic TME, (B) ECM remodeling may facilitate drug delivery and antitumor immune cell recruitment, and (C) targeting specific stemness biomarkers on CSCs or replicating under stemness promoters in CSCs to induce cytolysis. CSCs, cancer stem cells; ECM, extracellular matrix; TME, tumor microenvironment.

treatment induces the expression of CD80, CD86, and MHC II on CD cells, increases antitumor T cell infiltration while reducing the level of CD11b + Gr1+ MDSCs and FoxP3+ Tregs in TME of GBM mouse model, all improve the therapeutic effect of anti-PD-1 therapy upon their combination.[58] In addition to combination therapy, OVs such as oncolytic herpes simplex virus (oHSV) could be engineered to surface express full ICB or scFvPD-1 targeting immune checkpoint molecules (PD-1, CTLA-4, and PD-L1) and induce antitumor responses.[59] In addition, CSC-related signaling pathways such as epithelial–mesenchymal transition (EMT), STAT3, and AMPK induce upregulation of PD-L1 levels in CSCs, further highlighting the combination of ICB with many OV platforms (Table 3).

OVs also induce immunogenic cell death (ICD) through infection, replication, and lysis of both CSCs and tumor cells that are associated with the release of DAMPs molecules (e.g., ATP, HSP70, HSP90, HMGB1) and tumorigenic cytokines (IL-1b and IL-4), infiltration of T cells; all of which sensitize the tumor to ICB and CAR T cell therapy.[72–75] In addition, OVs induce the production of type I IFN in DCs, which mediates their activation for initiating T-cell priming and, for example, suppressing tumor cells with CSC properties in triple-negative breast cancer[76] and GBM.[77] OV-driven DAMPs generation also promotes DCs recruitment, maturation, and antigen presentation toward the activation of antitumor immune T cells,[78,79] which confirmed the potential of OV as a vaccine or robust the effect of DCs for tumor vaccination.

3.2 | OVs remodel tumor extracellular matrix (ECM)

The ECM is an integral part of the CSC niche and ECM receptors have been shown to facilitate CSC aggregation. Several stromal targeting approaches have been described using AdV to induce permanent ECM changes to improve therapeutic delivery. Oncolytic AdV expressing PH20 hyaluronidase was shown to destroy hyaluronan in the ECM, facilitating OV dissemination.[80] In addition, given the location of CSCs in the hypoxic core of the tumor mass and as hypoxia-driven MHC downregulation restricts T cell function, bi-specific T cell engagers (BiTEs) have been developed to target and kill tumor cells in an MHC-independent manner, however, BiTEs low penetration into the solid TME limited their efficacy.[81] To address this challenge, arming OVs with BiTEs[81] or OVs expressing the tumor-specific ECM remodeling factors such as MMP9 or hyaluronidase may improve the therapeutic efficacy and long-term animal survival in animal models (Figure 2B), particularly in combination with ICB therapy.[82,83] Similar to tumor cells, low or loss of TAA in CSCs as well as cold and immunosuppressive TME limited their targeting by CAR T/NK cells. Thus, the application of OV expressing CAR-specific TAAs or BiTEs may be able to present immunogenic TME and overcome the limitation of monotherapies with CAR T or BiTEs.[84,85]

TABLE 4 Examples of oncolytic viruses targeting CSCs of different sources.

Oncolytic virus	CSC source	Cancer type	Major finding	References
Adenovirus	Pleural effusion from breast cancer patients (CD44+CD24−/low cells)	Breast cancer	Reduced the proportion of CD44+CD24−/low and Hoechst excluding cells in those tumors	[86]
HSV-1 subtype NV1066	Stem-like tumor-initiating cells (TICs) tumorspheres	HCT8 human colon cancer	Treatment of TIC-induced tumors with NV1066 yielded tumor regression and slowed tumor growth	[87]
oHSV G47Δ	CSC-enriched cultures derived from human glioblastoma specimens	Glioblastoma	oHSV not only killed GBM-SCs but also inhibited their self-renewal in mice model	[88]
TRAIL-armed adenovirus ZD55	A549 cells derived-Tumorspheres	Lung cancer	Inhibited tumor growth and improved survival status of mice	[89]
Adenovirus OBP-301	Human MKN45/MKN7 cell-derived CD133+ CSCs	Gastric cancer	Decoyed quiescent CSCs in tumorspheres and xenografts model	[90]
GP73-regulated oncolytic adenovirus GD55	PLC/PRF/5 sphere cells	Liver cancer	Induction of remarkable apoptosis of liver CSC-like cells in vitro and in vivo, and inhibited the propagation of cells and angiogenesis in xenograft tumor tissues	[91]
HPV	CSC phenotype in HNC cell lines	Head and neck cancers	Improved clinical results observed in the HPV-positive status	[92]

Abbreviation: CSCs, cancer stem cells.

3.3 | OVs modulate stemness factor

Several OVs have demonstrated the potential to target and kill CSCs in various types of cancer (Table 4). Importantly, GBM-associated CSCs overexpress the adenoviral receptors that make them susceptible to infection by oncolytic adenovirus as Delta-24-RGD Ad showed improved ICD in GBM-CSCs xenografted model [93] and also long-term survival in a phase I trial of recurrent malignant glioma.[75] Bioinformatic analysis suggests that the expression of the Yamanaka pluripotency factors may be associated with the regulation of the selectivity of the OVs.[94] For example, such OVs such as Zika virus (ZIKV) showed specific tropism for infecting progenitor cells such as CSCs, and therapy with this type of OVs resulted in induction of SOX2-dependent apoptosis and decrease of CSCs population of CSC-derived orthotopic model.[95] Wnt/β-catenin signaling pathway is another pathway that supports CSC survival and invasion by promoting of EMT, that oncolytic adenovirus CD55-Smad4 showed induction of Smad4 expression, as an inhibitor of Wnt/β-catenin pathway, and thus suppressed the metastasis and stemness of CRC cells in vitro and a mouse xenograft model.[96]

Tumor-specific tropism of OVs could also be elicited by in vitro engineering of OVs that target specific CSCs or replicate under the promoter of overexpressing stemness factors (Figure 2C). For example, CSCs possessing multi-drug resistant induce therapy resistance in human cancers, and the application of tissue-specific promoter-driven oncolytic adenoviruses provides targeted OVs that efficiently target and kill CD44+CD24−/breast CSCs in vitro and in vivo.[97]

In addition, CSC are resistant to apoptosis post treatment and OV-expressing apoptosis inducer TRAIL can significantly kill the CSCs in vitro and in the GBM xenografted mouse model.[98,99] Oncolytic vaccinia virus lacking the small subunit of ribonucleotide reductase, F4L gene, more efficiently infect and lysis the ALDH + CD44 + CD24+ CSCs.[100] Also, OV expressing moieties targeting of CD133, EpCAM, and HER2 can further enhance tumor specificity[101] (Table 4 and Figure 2C).

4 | CHALLENGES FOR USING ONCOLYTIC VIROTHERAPY

Notably, despite the similarities between normal stem cells and CSCs, engineered OVs specifically kill CSCs while sparing healthy cells and stem cells. However, since not all OVs can affect CSCs from the same cancer, it is necessary to discover the unique mechanisms of each OV to kill tumor-specific CSCs and then design their therapies in combination with other therapies to target CSCs and surrounding niches (Table 3). These findings may pave a new way and provide an unprecedented opportunity for the combination of OVs with cancer immunotherapy.

Indeed, the efficacy of OV monotherapy is limited and challenged by the neutralization of treatment-induced or preexisting antiviral antibodies and cross-reactive antibodies,[102] the

rapid clearance of OV may be facilitated by mechanisms of antiviral resistance, including the activation of complement, antiviral cytokines, and phagocyte cells,[103,104] interstitial hydrostatic pressure, ECM, fibrosis, and necrosis may act as impenetrable physical barriers to OV entry,[83,105,106] and lack of biomarkers to assess the efficacy of OVs. Loss of viral fitness and reduction in replication competence and oncolytic activity may occur in OVs engineered for tumor selectivity.[107] These findings may pave a new way and offer an unprecedented opportunity for the combination of OVs with cancer immunotherapy.

Additionally, oncolytic virotherapy faces several challenges in preclinical and clinical trials. Murine cell lines are resistant to infection and replication by some OVs, and studies in immunocompetent murine models should be conducted under certain conditions.[108,109] Moreover, murine models may not mimic the human tumor biology and the interaction of OVs with the human immune system,[110] which could be improved using patient-derived xenograft murine models and patient-derived organoid models.[111,112] Clinical outcome of OV therapy has been limited by officious system that delivers the OVs to the tumor site while protecting them neutralization and dilution in the peripheral circulation, that developed nano-carriers can overcome the challenges associated with IV administration of OVs, as proposed and studied.[113] Overall, as a novel cancer therapy, oncolytic virotherapy, particularly in combination with ICIs, has shown potential in the treatment of solid tumors; however, various challenges continue to hamper the development of OVs, and there are many approaches being introduced to develop these therapeutic strategies that may significantly improve cancer-related outcomes.[114–116]

5 | CONCLUSION

Together, OVs induce ICD, activate APC, and (through these steps) then elicit adaptive T cell-dependent antitumor immunity against CSCs in and syngeneic the therapeutic effect of adoptive CAR T cell therapy. Thus, new insights into viral ontogeny and potential therapeutic techniques to target CSCs will contribute to the advancement of therapeutic approaches for cancer therapy, particularly in solid tumors where CSCs drive tumor progression and disease relapse. To selectively target CSCs, genetically engineered OVs are constructed using promoters, ICBs, ligands/receptors, and genes. The potential of multi-armed OVs to specifically target both tumor cells and CSCs and to remodel the immunosuppressive TME paves the way for the expansion of combination immunotherapies such as tumor vaccination, ICB and CAR cells, and engineered or modified OV platforms targeting specific CSC antigens or key pathways and their supporting niche in various types of solid tumors. However, the reversion of OVs into pathogenic form, sharing properties of CSCs with normal stem cells, and controlled delivery approach are just examples of challenges in their clinical application and thus need to be carefully considered.

AUTHOR CONTRIBUTIONS

Alborz Soroush, Reza Shahhosseini, Nima Ghavamikia, Nima Ghavamikia, Shahrzad Roudaki, Mahdi KhalatbariLimaki, and Mahtab Mirbolouk: Conceptualization; investigation; data collection; writing—original/revised draft; tables/figures preparation. **SeyedAbbas Pakmehr and Parvin Karimi**: Investigation; review and editing; visualization; supervision; and project administration. The authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Parvin Karimi http://orcid.org/0009-0004-4893-1709

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