Molecular Therapy
Oncology
Review

Emerging delivery strategy for oncolytic virotherapy

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Oncolytic virotherapy represents a promising approach in cancer immunotherapy. The primary delivery method for oncolytic viruses (OVs) is intratumoral injection, which apparently limits their clinical application. For patients with advanced cancer with disseminated metastasis, systemic administration is considered the optimal approach. However, the direct delivery of naked viruses through intravenous injection presents challenges, including rapid clearance by the immune system, inadequate accumulation in tumors, and significant side effects. Consequently, the development of drug delivery strategies has led to the emergence of various bio-materials serving as viral vectors, thereby improving the anti-tumor efficacy of oncolytic virotherapy. This review provides an overview of innovative strategies for delivering OVs, with a focus on nanoparticle-based or cell-based delivery systems. Recent pre-clinical and clinical studies are examined to highlight the enhanced efficacy of systemic delivery using these novel platforms. In addition, prevalent challenges in current research are briefly discussed, and potential solutions are proposed.

INTRODUCTION

Oncolytic viruses (OVs) represent a class of viruses capable of selectively infecting cancer cells either through a natural process or genetic modification, resulting in the targeted destruction of cancer cells, achieved through direct lysis or stimulation of anti-tumor immunity. Presently, only four OVs products (Rigvir, H101, T-Vec, and Delytact) have obtained clinical approval (Table 1).[1]

In 2015, T-Vec, a virus modified from oncolytic herpes simplex virus (HSV) and incorporating a segment of the granulocyte macrophage colony stimulating factor (GM-CSF) gene, demonstrated exceptional performance in the phase 3 OPTiM trial. This included significant advantages in durable response rate, improved progression-free survival, and overall survival,[2] leading to regulatory approval for the treatment of melanoma patients in stage III–IVM1a across various regions, including the United States, Australia, Israel, and Europe. Subsequently, researchers explored T-Vec as a neoadjuvant therapy for stage IIIB–IVM1a melanoma patients, yielding promising results in early clinical trials.[3] The success of T-Vec spurred the investigation of various viruses in clinical trial research, such as oncolytic vaccinia virus (VV), oncolytic measles virus (oMV), and reovirus (Table 2). Among these, Delytact emerges as a third-generation HSV. In a phase 2 clinical trial involving intratumoral injection for glioma patient treatment, Delytact demonstrated significant survival benefits and favorable safety profiles.[4] This led to its conditional and time-limited marketing approval in Japan in 2021, making it the first oncolytic virotherapy approved for primary brain tumor treatment worldwide. Furthermore, CG0070, a serotype 5 adenovirus (Ad) engineered to express GM-CSF, recently reported preliminary analysis results from a small-scale phase 3 clinical trial (NCT04452591). In this trial involving 66 patients with bladder cancer unresponsive to mainline treatment, CG0070 inhibited tumor growth in 64% of patients.[5]

Despite the achievements in OVs research, it is crucial to note that the prevailing delivery strategies for OVs predominantly rely on the local administration of naked viruses.[6] These local delivery methods encounter notable limitations, hindering widespread adoption in clinical settings. First, for widely spread metastatic tumors deep within the body, local injection methods struggle to completely eliminate tumor cells throughout the body. Second, the dense structure of most solid tumor tissues poses a challenge during intratumoral injection, as the high interstitial pressure may make it difficult for the virus to fully penetrate the tumor tissue.[7] Finally, intratumoral injection necessitates patients to exhibit good compliance and demands a high level of injection technique from health care professionals. While various studies explore emerging administration routes like arterial injection, intranasal administration, and high-pressure needle-free injection, the direct delivery of naked viruses faces challenges such as rapid clearance by anti-virus immunity, blockade by the reticuloendothelial system (RES), and severe side effects after systemic administration.[8] Therefore, alongside innovations in delivery routes, researchers actively investigate the utilization of novel drug delivery systems in OVs delivery studies. The goal is to enhance the targeting of OVs to tumor tissues and improve their diffusion within tumor tissues, even in the context of intravenous administration, addressing issues like rapid clearance and severe side effects.

https://doi.org/10.1016/j.omton.2024.200809.

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Table 1. Currently approved OVs worldwide

Virus family	Oncolytic agent	Administration route	Cancer	Location and time
ECHO	Rigvir	IM	melanoma	Armenia (2016), Georgia (2015), Latvia (2004)
Ad	H101	IT	nasopharyngeal carcinoma	China (2005)
HSV	T-Vec	IT	melanoma	USA (2015), Europe (2015), Australia (2016), Israel (2017)
HSV	Delytact	IT	glioblastoma	Japan (2021)

IM, intramuscular injection; IT, intratumoral injection.

Optimizing the delivery strategy for oncolytic virotherapy, addressing the aforementioned challenges, assumes a central role in oncolytic virotherapy research. Initially, several studies suggested that capsid modification of OVs (mainly oncolytic Ads [OAs]) could diminish the binding of viral receptors to normal tissues following systematic administration.[9–12] However, capsid modification alone is insufficient in effectively delivering OVs deep into tumor tissue resulting in limited anti-tumor effects.[13] Biological materials such as alginate, hydrogel, and silk-elastin-like protein polymer are used to shield OVs. OVs can be embedded in the material matrix through solid phase delivery. This shielding method enables controllable virus release, and localized gene expression in the surrounding environment after local administration.[14,15] However, this treatment is mainly suitable for local delivery and has restricted application, necessitating the exploration of more ideal OV delivery systems. This review concentrates on the research concerning nanoparticles (NPs) and cells as delivery carriers.

NP DELIVERY SYSTEMS

NPs, characterized by a particle size ranging from 1 to 100 nm, possess the ability to passively accumulate in tumor tissues through the enhanced permeability and retention (EPR) effect. The encapsulation of OVs with NPs can be achieved through physical interactions, such as enveloping OVs with liposomes and cationic polymers. Additionally, coating can be facilitated through chemical modification using polyethylene glycol (PEG) and arginine-grafted bioreducible

Table 2. Clinical trials on various OVs

Virus Family	Oncolytic agent	Administration route	Cancer	Phase	Status	Identifier
HSV	T-Vec	IT	melanoma	phase 3	completed	NCT00769704 NCT01368276 NCT02263508 NCT02297529
				phase 4	ongoing	NCT02910557
	OH2	IT	melanoma	phase 3	ongoing	NCT05868707
Ad	H101	IT	cholangiocarcinoma	phase 4	ongoing	NCT05124002
	ADV-TK	IT	prostate cancer	phase 3	ongoing	NCT01436968
	CG0070	IC	NMIBC	phase 3	ongoing	NCT04452591 NCT06111235
VV	JX-594	IT	HCC	phase 3	completed	NCT02562755
	GL-ONC1	IC	ovarian cancer	phase 3	ongoing	NCT05281471
Reovirus	Pelareorep	IV	SCCHN	phase 3	completed	NCT01166542
MV	MV-NIS	IC	ovarian cancer	phase 1	completed	NCT00408590
			pleural mesothelioma	phase 1	completed	NCT01503177
		IT	breast cancer, SCCHN	phase 1	completed	NCT01846091
		IV	myeloma	phase 2	completed	NCT02192775
Coxsackievirus A21	V937	IT	melanoma	phase 2	completed	NCT01227551
		IV	uveal melanoma	phase 1	completed	NCT03408587
H-1 Parvovirus	ParvOryx	IT/IV	glioblastoma	phase 1/2	completed	NCT01301430
MG1	MG1-MAGEA3	IV	NSCLC	phase 1/2	completed	NCT02879760
Poliovirus	Lerapolturev	IT	glioma	phase 1	completed	NCT03043391
NDV	MEDI5395	IV	solid cancer	phase 1	completed	NCT03889275

Results are only shown for partial clinical trials that use various OVs-based oncolytic virotherapy registered at ClinicalTrials.gov. The search of ClinicalTrials.gov was performed on February 17, 2024, using the following search terms: (oncolytic virus) AND (cancer) and filtered by clinical phase and trial status.
HCC, hepatocellular carcinoma; IC, intracavitary injection, including intravesical injection, intraperitoneal injection and intrapleural injection; IT, intratumoral injection; IV, intravenous injection; MV, measles virus; MG1, Maraba rhabdovirus; NDV, Newcastle disease virus; NMIBC, non-muscular invasive bladder cancer; NSCLC, non-small cell lung cancer; SCCHN, squamous cell carcinoma of the head and neck.

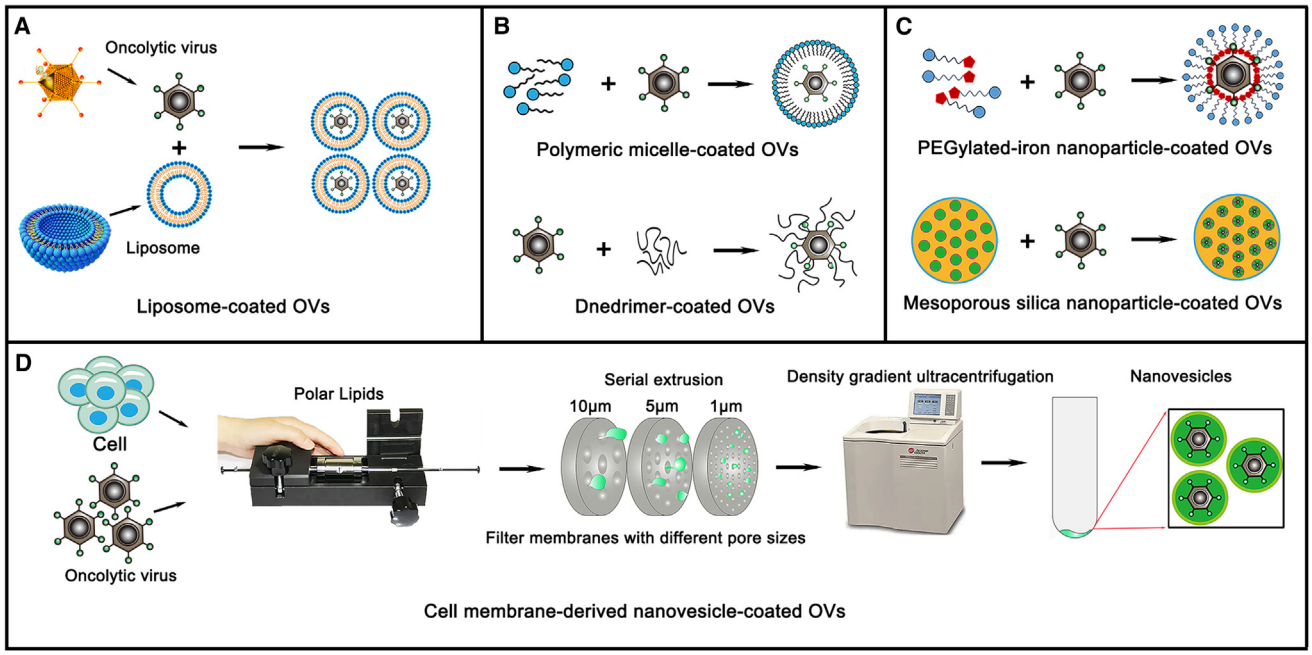

Figure 1. Schematic diagram of various nanomaterials encapsulating OVs

(A) Liposome-coated OVs, (B) polymer-coated OVs, (C) inorganic NP-coated OVs, (D) and cell membrane-derived nanovesicle-coated OVs.

polymer NPs.[16] Moreover, researchers have increasingly employed biomimetic nanomaterials, such as vesicles derived from cells, as carriers for viruses, with the goal of mitigating the immunogenicity of the carrier materials. The integration of NPs and OVs functions to impede the rapid clearance of OVs by the immune system through physical shielding and enhances tropism to tumor tissues via chemical modification (Figure 1).[17]

ORGANIC NPs

Liposomes, bioreducible vesicles characterized by a lipid bilayer primarily composed of phospholipids, have garnered significant clinical relevance.[18] Initially employed to shield OVs from neutralizing antibodies,[19] subsequent investigations have delved into the encapsulating OVs with liposomes for systemic delivery. This approach aims to enhance tropism and permeability to tumors, thereby augmenting viral genome transduction in tumors.[20–23] The direct encapsulation of the viral genome with liposomes has also been documented to decrease the size of material–viral complexes, optimizing passive tropism through the EPR effects.[24] Despite these advantages, liposomes encounter challenges like instability, burst release, and limited surface functionalization.

Polymers, long-chain molecules with excellent biodegradability, are widely used in drug delivery. A polymer is easy of synthesis and has excellent bio-degradability and bioavailability. Polymeric micelles and dendrimers, two main polymer NP types, provide flexible and controllable delivery capabilities. PEG, a representative polymeric micelle, is preferred for coating OVs due to its neutral charge and low immunogenicity.[25] PEGylation of Ads can be administrated by systemic way with prolonged cycle time, reduced uptake by macrophages and hepatocytes, and decreased antiviral immune response.[26–32] A recent report described the development of a lactose-PEG polymer (glycosylated-PEG) armed oncolytic HSV (glycosylated-PEG-oHSV) using a covalent conjugation method. This glycosylated-PEG-oHSV selectively infected and killed cancer cells by targeting the sialic acid glycoprotein receptor present in hepatocellular carcinoma cells.[33] Nevertheless, concerns have arisen regarding PEG's immunogenicity and potential hindrance to virus function.[34,35] Utilizing a cationic polymer as a carrier and complexing with OVs through electrostatic interaction can improve the virus's transduction rate to some extent.[36] Polyethyleneimine (PEI) is the most widely used dendrimer in gene delivery, belonging to cationic polymers. It can form complexes with OVs through electrostatic interactions. Encapsulating OVs with PEI for systemic delivery decreased the masking of tumor-specific ligands on the surface of OVs by polymeric micelles.[37–40] However, the high cationic density and poor degradability of PEI limit its applications.

To address limited specificity after systemic administration, OV-NP complexes were coupled with active targeting components like antibodies and peptides.[41,42] Tumor-specific peptides, including RGD and folic acid, improved nanocarrier delivery efficiency against cancer.[43–48] However, active targeting has limitations constrained by heterogeneous tumor characteristics.[49] Then, environmental responsive intelligent nanocarriers have emerged, offering enhanced precision, selectivity, and sensitivity in delivering OVs.[50] Researchers developed a pH-responsive polymer-coated OA; this hybrid carrier demonstrated superior targeted action, improved cellular uptake, and

anti-tumor efficacy under specific pH levels.[51–53] Additionally, external ultrasound further enhanced tumor tropism and penetration of viral nano-delivery platforms.[52]

INORGANIC NPs

Inorganic NPs, exemplified by iron and silicon dioxide, demonstrate synthetic scalability and robust chemical and thermal stability, rendering them widely applicable in diverse delivery contexts.[54]

Magnetic NPs (MNPs) find applications in diagnostics and biosensors.[55] Earlier research used MNPs to encapsulate OVs, enhancing their transduction into tumor cells under a magnetic field. This boosted OVs' anti-tumor efficacy when administered intratumorally.[56–58] A recent study synthesized a magnetized form of HSV1716, an OV has been under early clinical investigation, by combining it with nanomagnets from specialized magnetotactic bacteria. This magnetized OV, evading immune surveillance and utilizing magnetic targeting, increased virus accumulation at the tumor site, enhancing overall anti-tumor efficacy after systemic delivery.[59]

Silica materials have been shown to alleviate inflammation associated with intravenous Ad injection,[60] thus serving as gene delivery carriers. Researchers introduced silica onto OA surfaces with PEI, creating mineralization sites under mild conditions. Both *in vitro* and *in vivo* experiments demonstrated that silica-coated OA, after intravenous injection, had a longer circulation lifespan and notable anti-tumor effects compared with natural OA.[61]

In a recent study, researchers combined copper and manganese ions to prepare a multifunctional biomineralization coating for encapsulating OA surfaces. Compared with naked viruses, this composite effectively delivered the virus to tumor sites after intravenous injection and significantly inhibited tumor growth in a mouse subcutaneous tumor model. Additionally, copper and manganese ions also improved the tumor microenvironment.[62]

Despite their advantages, inorganic NPs face challenges such as low solubility and high toxicity, necessitating careful consideration for practical and clinical applications in virus delivery systems.[63]

CELL MEMBRANE-DERIVED NANOVESICLES

Cell membrane-derived vesicles have become a prominent focus in drug delivery research. Starting from the naturally secreted membrane vesicles of cells, such as exosomes and micro-vesicles, the research has advanced to the development of drug delivery technologies utilizing artificially manufactured exosome-mimetic or extracellular vesicles (EVs)-mimetic nanovesicles. This innovative approach mimics natural cell features, minimizing body clearance before reaching the target site. Leveraging the advantages of NPs, it serves as an ideal systemic carrier for OVs.[64]

Red blood cells (RBCs), abundant in blood, feature easily extractable and purifiable membranes. The CD47 protein on the RBC membrane inhibits the SIRP-α receptor, thereby reducing clearance of RBC membrane-coated NPs by the RES.[65] In recent years, researchers have engineered RBC membranes with tumor-specific ligands and pH-responsive properties separately to encapsulate OVs. These delivery platforms, through intravenous injection, effectively protected OVs from rapid clearance, thereby significantly enhancing tumor targeting.[66] Moreover, researchers have introduced hybrid membrane vesicles, combining artificial lipid membranes and RBC membranes, for improved surface antigen masking and extended OVs circulation time.[67]

The membrane of tumor cells possesses various unique characteristics, including immune evasion, resistance to apoptosis, and prolonged circulation time, rendering it a suitable source for biomimetic nanocarrier systems. Additionally, tumor cell membranes inherit the antigen repertoire of their parent cells, enabling their utilization in tumor-targeted therapy and immunotherapy.[68] Subcellular vesicles from tumor cells can carry OVs. After intraperitoneal injection, these vesicles exhibited a potent anti-tumor effect, even in malignant ascites models, characterized by strong immune suppression.[69] Furthermore, EVs derived from tumor cells can co-load OAs and chemotherapeutic drugs, enhancing anti-tumor effects after intravenous injection.[70] The study team substantiated the specific tumor-targeting capability of these EVs formulations through the use of bioluminescence and fluorescence imaging techniques, confirming their ability to target tumors.[71] Recently, Another team of researchers modified Coxsackie virus B3 (CVB3) with microRNA and generated exosomes carrying CVB3 (ExomiR-CVB3) after infecting tumor cells. Subsequently, ExomiR-CVB3 underwent a two-step modification process involving encapsulation of the chemotherapeutic drug doxorubicin (Dox) and decoration with the AS1411 aptamer. The final therapeutic platform ExomiR-CVB3/DoxApt demonstrated improved targeting and cytotoxic capabilities against subcutaneous breast cancer in mice after intraperitoneal injection.[72] In addition to using naturally secreted cell particles, some studies have reported the generation of artificial enveloped viruses by co-extruding OAs with cancer cell membranes. This method not only preserved the virus's activity, but also enhanced its anti-tumor capabilities after intratumoral injection.[73] By engineering OAs to express the programmed cell death 1 extracellular domain, the artificial enveloped virus system maintained strong anti-tumor effects, even in the immunosuppressive environment of liver cancer ascites after intraperitoneal injection.[74]

Additionally, a study has demonstrated that EVs from neural stem cells (NSCs) and glioblastoma cells could coat OA, and both types could penetrate the blood-brain barrier and target tumor cells in the brain after intravenous injection.[75] Beyond cell membranes from homologous sources, researchers have proposed the concept of a microbial nanocomplex. They encapsulated modified OVs within bacterial outer membrane vesicles, resulting in a self-amplifying cascade-enhanced anti-tumor immune therapy after intravenous injection.[76]

Utilizing cell membrane-derived particles for delivering OVs still presents some challenges. The spike-like structure of OVs can pierce lipid bilayers, exposing abundant antigens easily recognized by

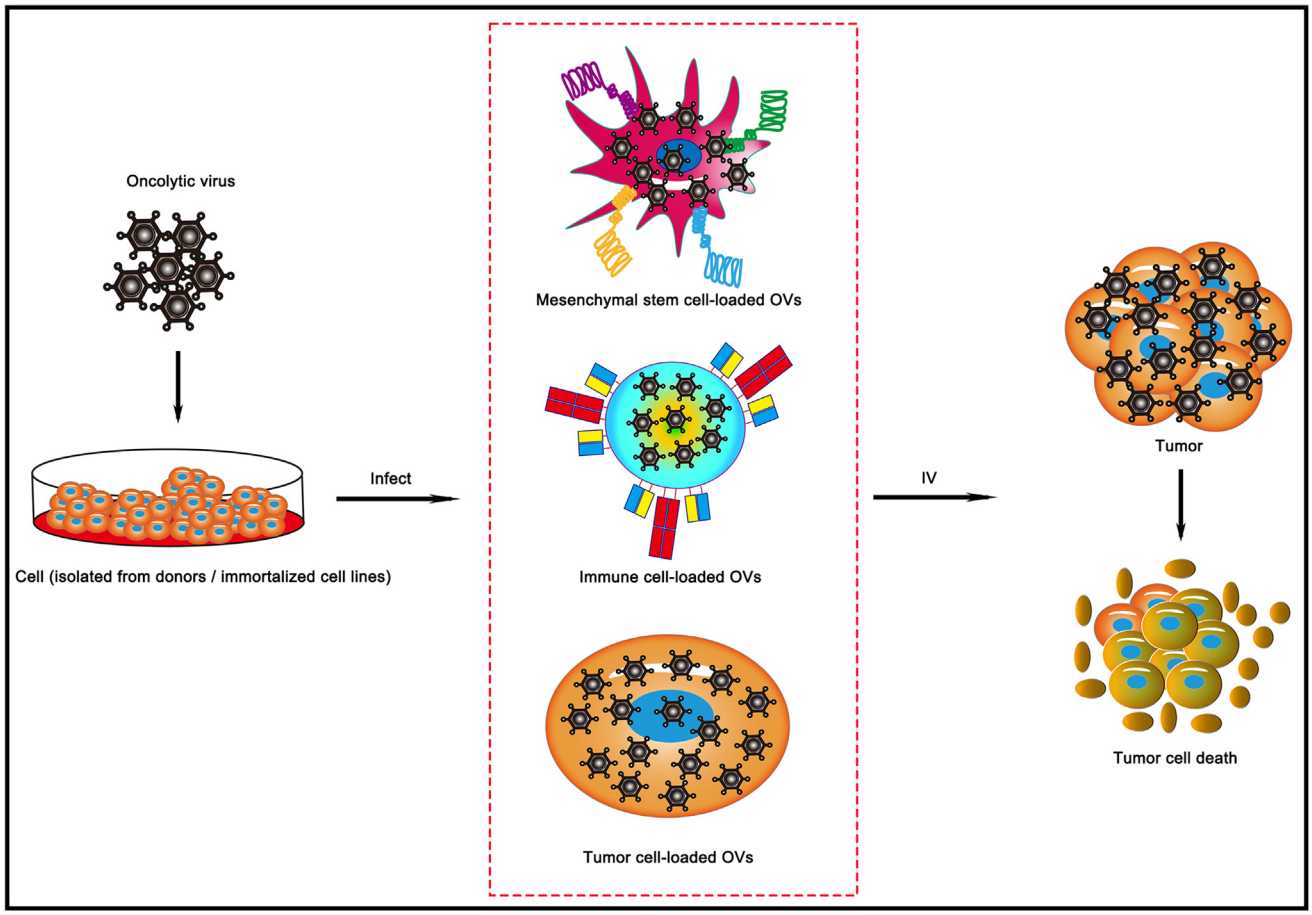

Figure 2. Schematic diagram of various cells loading OVs

Toll-like receptors on the surface of immune cells, leading to rapid viral clearance.[77,78] Therefore, the cell membrane employed to encapsulate OVs requires enhanced coverage and integrity. Furthermore, cell membranes coating OVs need to have higher coverage and integrity. Adding cholesterol or utilizing hybrid membranes could both aid in stabilizing these nanovesicles.[67,79]

CELL-BASED DELIVERY SYSTEMS

In comparison with NP drug delivery systems, cell-based drug delivery systems offer various advantages, including prolonged drug circulation, enhanced efficacy, controlled drug release, and limited immunogenicity and cytotoxicity.[80] Notably, SCs and immune cells have notably undergone extensive investigation as delivery systems for bioactive drugs due to their inherent affinity for diseased tissues.[81–83] In the context of delivering OVs, cell carriers present a distinctive advantage by serving as an ideal virus production factory (Figure 2).

SCs

SCs constitute a class of cells endowed with the capacity for self-renewal and differentiation into specialized cell types. These cells possess potential immunomodulatory properties, contribute to the regeneration and repair of damaged tissues, and have been central to cell carrier research.[84] Homing stands out as a crucial characteristic of SCs, essential for their effective clinical application.[85,86] SCs serve as a promising vector for OVs,[87] facilitating targeted viral delivery to tumor sites, shielding the virus from the RES, and providing protection against elimination by systemic or local antiviral immune responses.[88] Current research in the field of SCs as carriers for OVs focuses primarily on mesenchymal SCs (MSCs) and NSCs.

MSCs, characterized as pluripotent SCs capable of differentiating into restricted or lineage-specific cell types, represent the first generation of clinical SCs.[87] Commonly derived from bone marrow and adipose tissue,[89] MSCs have seen approval for several clinical applications.

Earlier studies revealed that a modified viral capsid of OA enhanced viral infection and replication in MSCs. They demonstrated these MSCs shielded-OA effectively targeted to tumor in many xenografted mouse models after local or systemic administration.[90–99] A study demonstrated that, after intra-arterial injection, human-derived MSC-loaded OA DNX-2401 can selectively deliver the virus to tumor

Table 3. Clinical trials on using SC-loaded OVs

Virus family	Oncolytic agent	SC type	Administration route	Cancer	Phase	Status	Identifier
Ad	ICOVIR5	MSC	IV	solid cancer	phase 1/2	completed	NCT01844661 [103]
			IV	uveal melanoma	phase 1/2	ongoing	NCT05047276
			IV	DIPG	phase 1	ongoing	NCT04758533
	CRAd-S-pk7	NSC	IC	glioma	phase 1	completed	NCT03072134 [120]
			IC	glioma	phase 1	ongoing	NCT05139056
			IC	glioma	phase 1	ongoing	NCT06169280
	DNX-2401	MSC	IA	glioma	phase 1	ongoing	NCT03896568 [101]
MV	MV-NIS	MSC	IP	ovarian, peritoneal or fallopian tube cancer	phase 1/2	ongoing	NCT02068794

Results are only shown for clinical trials on SC-loaded OVs registered at ClinicalTrials.gov. A preliminary search of ClinicalTrials.gov was performed on February 17, 2024, using the following search terms: (oncolytic virus) AND (cancer). After the preliminary search, the names of identified agents were then used for a secondary search.
IA, intra-arterial injection; IC, intra-resection cavitary injection; IP, intraperitoneal injection; DIPG, diffuse intrinsic pontine glioma.

site of glioma in mice and exert oncolytic effects.[100] Recently, the same team has demonstrated that perfusion-guided endovascular super-selective intra-arterial infusion of MSC-loaded DNX-2401 was safe in humans.[101] Garcia-Castro's team first studied clinical application of CELYVIR (X-ray irradiated autologous MSCs carrying OA ICOVIR-5) in the treatment of four children with refractory metastatic neuroblastoma. They demonstrated the safety of this treatment regimen. One child was observed to be in complete remission for 3 years after treatment.[102] A recent phase 1 clinical trial conducted by the same team has expanded the sample size and recruited patients with various solid cancer, further confirmed the safety of CELYVIR via intravenous administration.[103] The team also verified in pre-clinical research that both isogenic and allogeneic CELYVIR activated the immune system after intraperitoneal injection.[104] Additionally, studies have also reported that systemic administration of MSC-loaded oMV enhanced tumor tropism and anti-tumor efficacy on various tumor models.[105–107] And there is a phase 1 clinical trial for evaluating the MSC-loaded oMV ongoing (Table 3). Studies also demonstrated patient-extracted MSCs did not compromise their function as therapeutic vectors for OVs.[108,109] Moreover, MSCs have been reported to carry HSV, myxoma virus, NDV, VV, and other viruses.[110–117] To further enhance the tumor tropism of OVs-carrying MSCs, researchers improved the procedure such as using OVs expressing therapeutic genes, or in combination with MSCs expressing cytokines.[118,119]

NSCs are cells derived from or differentiated into neural tissues. NSCs have found widespread applications in the treatment of brain tumors due to their neuroprotective and neurotrophic functions.[121,122] Autologous transplantation of NSCs is not clinically feasible. As an NSC cell line that has been approved by the U.S. Food and Drug Administration for the treatment of recurrent glioma, HB1.F3-CD cell has been utilized to deliver OVs in many pre-clinical studies. In addition, induced NSCs offered a promising approach to use single-factor *trans*-differentiation to make patient-specific tumor homing cells derived from fibroblasts.[123] Breakefield 's team has demonstrated for the first time that NSCs can be used to deliver OVs, which expended the viruses' infection area after local administration.[124,125] Lesniak's team confirmed that NSCs loaded with OA significantly enhanced virus spread at the tumor site and prolonged survival after intracranial tumor injection, both in immunodeficient and immunocompetent mouse models.[126–130] Based on these pre-clinical experimental data, the team conducted a corresponding phase 1 clinical trial, demonstrating the safety and effectiveness of the therapeutic vector after intracranial injection in humans.[120] Furthermore, combining NSC-shielded OVs with other therapies or utilizing engineered NSCs has shown promise in enhancing efficacy after local administration.[131–133] Feasibility studies have also verified the potential of delivering NSC-based OV systems through nasal administration.[133,134]

Recently, research has employed human dental pulp-derived MSCs (hDPSCs) from wisdom teeth as carrier cells for OVs. Compared with other SC types, hDPSCs are easier to obtain, possess a strong proliferation capability, and secrete various growth factors, rendering them more suitable for clinical use. The focus has been on the OA YSCH-01, currently undergoing clinical development. Results showed that intraperitoneal injection of YSCH-01/hDPSCs effectively targeted the tumor site. Moreover, the viral load carried by hDPSCs was only one-tenth of that administered via intratumoral injection, yet achieved a therapeutic effect comparable to that of directly injected naked virus.[135]

IMMUNE CELLS

Immune cells do not elicit adverse immune responses; they exhibit superior biocompatibility, minimal interaction with normal cells, and the ability to actively target specific cells and sites. Consequently, immune cells present a promising avenue for drug delivery systems. Employing cytotoxic immune cells to carry OVs serves a dual purpose as a Trojan horse and as agents capable of directly eliminating tumor cells.

T cells constitute the principal cells of the adaptive immune response. A prior investigation indicated that by isolating autologous non-antigen-specific T cells from blood and co-culturing them with oncolytic

vesicular stomatitis virus (VSV), VSV could adhere to the T cell surface, creating a T cell-OV drug delivery system. Upon intravenous injection, this system selectively eradicated metastatic lesions in lymph nodes.[136] In contrast to the surface connection between OVs and T cells, dendritic cells (DCs) shielded OVs through internalization, rendering them superior delivery vehicles for OVs. Reovirus loaded by mature DCs could more efficiently target tumors following intravenous administration.[137,138]

Monocytes and macrophages excel as cell carriers owing to their capacity to respond to hypoxia and inflammation, distribute uniformly within tumors, display robust phagocytosis, and offer easy accessibility from peripheral blood. Russell's research team documented that monocytes infected with oMV, including the monocyte cell line U-937 and primary human $CD14^+$ cells, could effectively transport the virus to tumor lesions via intraperitoneal or intravenous injection.[139,140] Monocytes also served as effective vehicles for the systemic delivery of oncolytic HSV.[141] Furthermore, macrophages derived from monocytes loaded with hypoxia-regulated OA can precisely target delivery to tumor sites after intravenous administration, resulting in a significant inhibition of tumor growth at both primary and metastatic lesions.[142]

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells that promote immune tolerance within the tumor microenvironment. They dynamically migrate to the tumor site from the circulation, guided by the recognition of the tumor's microenvironment through their surface receptors. Serving as carriers, they facilitated the transportation of VSV with precision, targeting tumors effectively upon systemic administration. Additionally, VSV infection induced MDSC differentiation toward a classically activated M1-like phenotype.[143]

Cytokine-induced killer (CIK) cells represent a novel class of immunologically active cells, demonstrating robust anti-tumor activity similar to T lymphocytes and leveraging non-major histocompatibility complex-restricted tumor-killing advantages similar to natural killer (NK) cells.[144] Studies demonstrated the ability of CIK cells to target various tumors, exhibiting anti-tumor efficacy in both murine and human subjects.[145] Pioneering research has explored the utilization of mouse or human CIK cells loaded with oncolytic VV or extracellular enveloped virus (EEV) derived from poxviruses. Systemic injection in mouse models achieved targeted delivery of VV/EEV, thereby enhancing their anti-tumor effects. Through virus engineering, the targeting capability of CIK cell carriers toward tumors was augmented by the expression of specific chemokines.[146,147]

NK cells are highly cytotoxic immune effector cells with the ability to transform the suppressive immune microenvironment into an inflammatory one. They exhibit excellent homing ability and serve as effective carriers for OVs.[148] Recent research has demonstrated the feasibility of using NK cells to load OA (Ad@NKs), achieving efficient systemic tumor-targeted delivery. Furthermore, upon Ad infection of NK cells, the enhancement of NK cell functionality occurred through the upregulation of Type I interferon signaling. Both *in vitro* and *in vivo* data indicated that Ad@NKs can disrupt tumor cells, induce immunogenic cell death, and improve the immune microenvironment, exhibiting excellent anti-tumor and anti-metastatic capabilities.[149]

Numerous studies have highlighted synergistic effects between chimeric antigen receptor-T cell (CAR-T) immunotherapy and OVs, particularly in solid tumors. This synergy originates from the pre-administration of OVs, which converts the cold tumor microenvironment into a hot one, thereby establishing a conducive milieu for CAR-T functionality.[150] In 2022, a groundbreaking study pioneered the utilization of CAR-T cells loaded with OVs. Upon the systemic delivery of this platform, it not only enhanced the tumor targeting of OVs, but also prolonged the survival of tumor-bearing mice.[151]

Currently, the majority of studies employing immune cells as carriers for OVs are in the proof-of-concept stage and have not undergone clinical validation.

TUMOR CELLS AND OTHERS

Early studies have demonstrated the potential of various cancer cell lines, infected with diverse OV types, for delivering OVs. Upon systemic delivery, OVs can effectively reach the tumor site, as evidenced by prior investigations.[152–154] Nevertheless, cells derived from solid tumors would encounter interception in the lungs. When leukemia cell lines, such as murine-derived L1210 or human-derived U-937 cells, were employed, OVs exhibited improved delivery to distant subcutaneous tumors after intravenous injection.[139,155] The application of transient immunosuppression with immunosuppressive agents before the injection of cell delivery system further enhanced the efficiency of tumor carriers.[156] After infection, tumor cells may retain potential tumorigenicity. To address this, some researchers subjected carrier cells to irradiation before utilizing them as a platform for injecting tumor cells, suppressing the activity of these cells.[152] However, irradiated tumor cells may lose their ability to produce OVs. Therefore, a recent study has proposed the use of liquid nitrogen shock treatment on tumor cells to eliminate the pathogenicity of the tumor cell carrier while preserving its infectivity and activity.[157]

Additionally, studies have reported that immortalized human peritoneal mesothelial cell lines and immortalized human hepatic stellate cell lines can function as effective delivery carriers for OVs. Moreover, they can safely and efficiently deliver OVs through intraperitoneal injection or intranasal administration.[158,159]

NP-CELL INTEGRATED DELIVERY SYSTEMS

The most appealing advantage of cell carriers is their role as a production factory for viruses. Currently, research utilizing SCs as carriers for delivering OVs has advanced to early-phase clinical trials, confirming the safety of the delivery system. However, cell carriers also have some drawbacks, including poor active targeting and susceptibility to virus infection by various cell carriers. Consequently,

Table 4. Characteristics of OVs carriers

Type	Regular NP	Cell	Cell membrane-derived vesicle
Preparation	mostly easy	relatively easy for immortalized cell lines difficult for extracted terminal cells	relatively difficult compared with regular NP
Immunogenicity	relatively high	very low	very low
Tumorigenicity	no	certain risk for immortalized cell lines	no
Natural tropism	EPR effect	mostly rely on the crosstalk between receptors and ligands	with the integrated properties of NPs and cells
Production of OVs	no	yes	no
Clinical trials	none	see Table 3	none

researchers actively integrate cell carriers with nanotechnology to address these limitations (Table 4).

First, nanomaterials can be utilized to modify OVs. During the preparation of OV-cell delivery systems *in vitro*, nanomaterial modification enhances the internalization efficiency of OVs into host cells. Studies have included complexing OAs with biodegradable polymers, and others have utilized PEGylated gold NPs as a coating formulation, both of which significantly increased the infection efficiency of OAs for MSCs. In mouse tumor models, the improved treatment platform exhibited superior anti-tumor efficacy compared with MSC carriers without nanomaterial coating.[160,161]

Second, nanomaterials can be utilized to remodel pre-prepared OV-cell delivery systems. During the circulation phase of the delivery system, the addition of nanomaterials can enhance the tumor targeting of cell carriers. One method of targeted magnetic cell delivery is magnetic resonance targeting (MRT), utilizing the inherent magnetic field gradient of an magnetic resonance imaging system to guide magnetic particles to deep target areas.[162] Previous studies have reported the infection of OAs into macrophages derived from peripheral blood, followed by co-incubation with superparamagnetic iron oxide NPs (SPIOs), resulting in a macrophage delivery system containing SPIOs and OAs. The use of MRT increased the accumulation of this delivery platform in primary and metastatic tumors in mice after intravenous injection.[163] The addition of MNPs, besides bringing therapeutic benefits, also served as a non-invasive monitoring tool for the virus delivery platform.[134,164] Recently, research has modified the 293T-OV delivery platform by asymmetric immobilization of Fe_3O_4 NPs on the cell surface. After intravesical injection, the asymmetric coating of MNPs and the targeting molecule cRGD peptide enabled cell carriers to migrate directionally to the tumor site under magnetic control.[165]

Additionally, recent studies have developed new delivery strategies by directly connecting OVs modified with nanomaterials to live cells. These approaches leverage viable cellular mechanisms to enhance OVs' active targeting toward tumors and exploit nanomaterials to augment OVs' transduction into tumor cells. One investigation employed positively charged PEI to facilitate the binding between negatively charged OVs and RBCs, resulting in the development of RBC leveraged OV therapy (ELeOVt). ELeOVt extended the circulation time of OVs after intravenous injection and boosted the transduction efficiency of OVs into tumor cells, demonstrating notable therapeutic efficacy in a murine cancer model with lung metastasis.[166] Other research has reported a chimeric entity of engineered OAs and tumor-specific T cells (including T cell receptor T and CAR-T cells). First, OAs were encapsulated within cell membranes containing tumor-specific antigens (including tumor cells and engineered 293T cells) through lipid squeezing to obtain M@eOA. Subsequently, M@eOA was anchored on the surface of T cells. Intravenous administration of this therapeutic platform significantly prolonged survival in mouse orthotopic tumor models and humanized mouse tumor models, while also improving the tumor microenvironment.[167]

CONCLUSION

Both nanomaterials and cells function as delivery vehicles for OVs, each with a lengthy research history that has yielded numerous promising pre-clinical results. However, only studies employing SCs as delivery systems have progressed to early clinical research stages and achieved promising outcomes. We posit that breakthroughs in translating this research can be realized from several perspectives. First, leveraging the advantages of different delivery carriers and designing more rational delivery strategies tailored to different cancer types. Second, simplifying the composition of delivery vehicles to decrease the complexity of constructing OVs delivery platforms can enhance stability and safety. Last, integrating tracing materials and non-invasive monitoring methods can further elucidate the pharmacokinetics of OVs delivery platforms *in vivo*. By integrating these approaches with advanced techniques, such as single-cell sequencing, we can comprehensively unravel the mechanisms underlying the therapeutic effects of OV delivery platforms, thereby establishing a more robust theoretical foundation for advancing this field.

ACKNOWLEDGMENTS

This study was funded by the Natural Science Foundation of Sichuan, China (24NSFSC1306), the National Natural Science Foundation of China Program grant (82273315), and the 1.3.5 project for disciplines of excellence, West China Hospital, Sichuan University (ZYGD23023).

AUTHOR CONTRIBUTIONS

J.Z. conceived and drafted the paper; J.M. and M.H. conducted literature reviews and created illustrations; G.S. and H.D. primarily contributed to the conceptualization and revision of the review, overseeing the project. All authors contributed to the writing, editing, and reviewing of this manuscript.

DECLARATION OF INTERESTS

The authors declare that there are no conflicts of interests.

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