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Therapeutic potential of oncolytic viruses in the era of precision oncology

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ABSTRACT

Oncolytic virus (OV) therapy has been shown to be an effective targeted cancer therapy treatment in recent years, providing an avenue of treatment that poses no damage to surrounding healthy tissues. Not only do OVs cause direct oncolysis, but they also amplify both innate and adaptive immune responses generating long-term anti-tumour immunity. Genetically engineered OVs have become the common promising strategy to enhance anti-tumour immunity, safety, and efficacy as well as targeted delivery. The studies of various OVs have been accomplished through phase I-III clinical trial studies. In addition, the uses of carrier platforms of organic materials such as polymer chains, liposomes, hydrogels, and cell carriers have played a vital role in the potentially targeted delivery of OVs. The mechanism, rational design, recent clinical trials, applications, and the development of targeted delivery platforms of OVs will be discussed in this review.

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Introduction

Cancer is a major public health problem worldwide, accounting for almost 10 million cancer deaths and over 19 million new cancer cases in 2020.[1] In 2022, the estimated numbers of new cancer cases and deaths in the United States are 1,918,030 and 609,360 people, respectively.[2] Chemotherapy is a major therapeutic approach to cancer treatment. However, success of chemotherapy has been limited due to a lack of selectivity toward cancer cells, rapid drug metabolism, and multidrug resistance, mainly resulting from increased efflux pumps in the cell membrane which transport various anti-cancer drugs out of the cells.[3,4] In addition, conventional chemotherapy may significantly damage healthy cells causing harmful side effects in patients.[5,6]

Oncolytic virus (OV) therapy has recently been recognised as a promising new therapeutic strategy in cancer treatment, which can circumvent some drawbacks of conventional chemotherapy. Due to their ability to specifically target and lyse tumour cells without harming surrounding healthy cells, as well as induce anti-tumour effects by multiple action mechanisms, OV can decrease the emergence of acquired drug resistance.[7-9] In addition to direct oncolysis, it can also amplify both innate and adaptive immune responses generating long-term anti-tumour immunity.[9-11] The targeted delivery and anti-tumour immunity are critical for potential applications of OVs in cancer therapy. However, a key challenge facing oncolytic virotherapy is the anti-viral immune responses from vaccinated patients, which may lead to viral clearance that limits the overall therapeutic efficacy of OVs. Thus extensive research efforts have been aimed to engineer OVs for improving their efficacy, safety, tumour-specific targeting, viral delivery, and anti-tumour immune evasion.[12,13] The literature retrieval strategy of this review is shown in **Additional file 1**.

Mechanism and Rational Design of Oncolytic Viruses

OVs utilise several mechanisms to preferentially enter and replicate in cancer cells. Many OVs have a natural tropism for the surface receptors

that irregularly overexpressed on cancer cell surfaces such as CD46, CD54, CD55, CD155, laminin, integrin α2β1, etc.[13-15] OVs can utilise and recognize those receptors to enter the targeted cancer cells as shown in **Table 1**. For example, adenovirus utilises coxsackievirus and adenovirus receptor (CAR) as a primary receptor to ensure attachment, and cell surface integrins (αvβ3 and αvβ5) to further facilitate viral internalization.[16] CAR expression is upregulated in basal cell carcinoma, thyroid adenoma, lung, ovarian, cervical, and laryngeal cancer cells.[17] Herpesvirus utilises herpesvirus entry mediator known as tumour necrosis factor receptor superfamily-14 for cell entry, which is overexpressed in breast cancer, gastric cancer, and hepatocellular carcinoma.[18, 19] Parvovirus H1 (H-1PV) utilises sialic acid residue presented on laminin for cell binding and enters cells via clathrin-mediated endocytosis.[20,21] In addition, a recent study found that galectin-1 also plays a key role in the cell entry of H-1PV.[22] H-1PV shows anti-cancer activity toward a variety of cancers, such as glioma, melanoma, pancreatic, breast, lung, cervical, and colon cancer.[23, 24] Coxsackievirus utilises CD54 and CD55 receptors as the primary and secondary points of viral attachment and internalization, which are overexpressed in malignant glioma, myeloma, melanoma, head and neck, lung, colon, pancreatic, and breast cancer cells.[25, 26] Poliovirus utilises the CD155 receptor, which is overexpressed in colorectal carcinoma, glioblastoma, melanoma, sarcoma, hepatocellular carcinoma, non-small-cell lung carcinoma, and pancreatic cancer cells.[27-30] Measles virus utilises CD46 receptor which is overexpressed in some cancer cells such as myeloma, hepatocellular carcinoma, colorectal, prostate, ovarian, and breast cancer cells.[14, 31] Normally, CD46 protects healthy cells from cell elimination by complement attack.[31] Vesicular stomatitis virus (VSV) utilises low-density lipoprotein receptor as a major receptor and other low-density lipoprotein receptor family members as alternatives for attachment and cell entry.[32, 33] Cancer cells that are susceptible to VSV are glioblastoma, melanoma, osteosarcoma, hepatocellular carcinoma, breast, cervical, and pancreatic cancer cells.[34] Sindbis virus enters cells through laminin receptor, which is overexpressed in uterine adenocarcinoma, melanoma, colorectal carcinoma, breast carcinoma, and non-small cell lung carcinoma.[35-39] Echovirus utilises integrin α2β1 as its cellular receptor for cell entry, which is overexpressed in ovarian, prostate, and gastric cancer cells.[40, 41] Newcastle disease virus binds to a sialic acid receptor for virus attachment to host cells, which demonstrates anti-cancer activity toward glioma and melanoma, renal cell carcinoma, ovarian, and cervical cancer.[42-45]

Table 1. The cell entry receptor and the aberrant oncogenic signalling pathway that OVs utilise to preferentially enter and replicate in cancer cells

Virus					
Genome type	Name of the OV	Enveloped	Cell entry receptors	Aberrant oncogenic signalling pathway	References
DNA	Adenovirus	N	CAR, integrins	PKR, Rb and p16	14, 16, 46
	Herpesvirus	Y	HVEM	PKR, Rb and p16	14, 18, 19, 46
	Parvovirus H1	N	Sialic acid, galectin-1	–	20-22
	Vaccinia virus	Y	–	RAS, PKR, Rb and p16, IFN-1	14, 46
RNA	Coxsackievirus	N	ICAM-1 (CD54), DAF (CD55)	–	25, 26
	Poliovirus	N	CD155	–	27-30
	Measles virus	Y	CD46	–	14, 31
	Vesicular stomatitis virus	Y	LDLR	IFN-1	14, 32, 33
	Sindbis virus	Y	LAMR	–	35-39
	Echovirus	N	Integrin a2b1	–	40, 41
	Reovirus	N	–	RAS, PKR, Rb and p16	14, 46
	Newcastle disease virus	Y	Sialic acid	Bcl-xL, IFN-1	14, 44-46, 57

Note: Bcl-xL: B-cell lymphoma-extra large; CAR: coxsackievirus and adenovirus receptor; DAF: decay-accelerating factor; HVEM: herpesvirus entry mediator; ICAM-1: intercellular adhesion molecule 1; IFN-1: type I interferon; LAMR: laminin receptor; LDLR: low-density lipoprotein receptor; N: no; p16: tumour suppressor protein; PKR: protein kinase R; RAS: rat sarcoma; Rb: retinoblastoma; Y: yes.

Furthermore, OVs exploit aberrant signalling pathways and can replicate in tumour cells, which have defects in anti-viral pathways.[13,14] Cancer cells promote their survival, proliferation, and metastasis by manipulating cellular transcriptional and signalling pathways.[46] Additionally, cell cycle and cell proliferation in tumour may be disrupted by oncogenes and the deficit of tumour suppressor genes, which allows OVs to survive longer in cancer cells.

In healthy cells, the natural cellular defense mechanisms in response to viral infection, including interferon (IFN) and protein kinase R (PKR) signalling pathways, induce infected cells to undergo apoptosis and viral clearance.[14] Type I IFN (IFN-1) is a cytokine in an early host defense that occurred prior to the immune response and possesses anti-viral activity.[47-49] Stimulating the release of IFN-1 during the viral infection triggers the intracellular signalling pathway, mainly

Janus kinase-signal transducers and activators of transcription pathway, and eventually limits viral replication and enhances the rate of viral clearance and the immune responses in infected cells.[47] Additionally, the activation of IFN-1 pathway induces PKR expression and activation.[50] PKR is a major host defense against viruses and can be activated by viral-specific RNAs.[47] The activated PKR phosphorylates the eukaryotic translation initiation factor 2, resulting in the inhibition of protein translation and synthesis, and consequently suppressing viral replication and spreading.[51] PKR also involves in cellular differentiation, proliferation, and apoptosis.[52]

In contrast, PKR and IFN in regulating viral clearance on cancer cells are impaired.[50] Rising metabolic activity of cancer cells also enhances viral replication and the rate of cell lysis compared to healthy cells.[53] These aberrant signalling pathways of carcinogenesis render cancer cells susceptible to viral infection, which is summarised in **Table 1**. Some OVs naturally exploit the aberrant expression of various proteins usually involved in the rat sarcoma (RAS) pathway. For example, the suppression of p16, a tumour suppressor, together with over-active RAS signalling in cancer cells can influence the expression of retinoblastoma and prevent it from regulating cell cycle entry and restricting cell proliferation.[54-56] In addition, over-active RAS signalling in cancer cells can inhibit PKR signalling and block cell apoptosis.[14] It has been identified that vaccinia virus and reovirus selectively target various tumours with activation of RAS signalling.[46] Some viruses such as adenovirus, herpesvirus, vaccinia virus, and reovirus utilise the defects in cell cycle regulation and anti-viral mechanisms in tumours such as PKR, retinoblastoma and p16 for viral replication and survival.[14, 46] In addition, dysregulation of IFN-1 pathway in cancer cells, which plays an important role in anti-viral and anti-tumour responses, facilitates some viruses such as vaccinia virus, VSV, and newcastle disease virus to replicate preferentially within tumours.[14] Cancers often overexpress anti-apoptotic molecules such as B-cell lymphoma (Bcl) family of proteins for cell immortality, and newcastle disease virus targets Bcl-xL-overexpressing cells, promoting viral accumulation and replication.[46, 57] OVs use the cellular machinery for their replication and protein production, while affect cell functions, stimulate oxidative stress, and activate the pathways involving autophagic process.[48]

Not only do OVs lyse cancer cells leading to tumour regression but also concurrently create and stimulate anti-tumour immunity, resulting in eradication of the disease and prevention of the recurrence.[46, 58, 59] **Figure 1** shows the anti-tumour activity of OVs associated with multiple mechanisms involving inflammation process and immunogenic cell death.[59] Following the cancer cell damage by OV-induced oncolytic cell death, viral progeny such as pathogen-associated molecular patterns and immune signs such as damage-associated molecular patterns are produced and released, which will stimulate the maturation of dendritic cells and promote the release and the expression of tumour-associated antigens and tumour-associated neoantigens to the immune cells and cancer cells.[14, 48] Antigen presentation through major histocompatibility complex class II and major

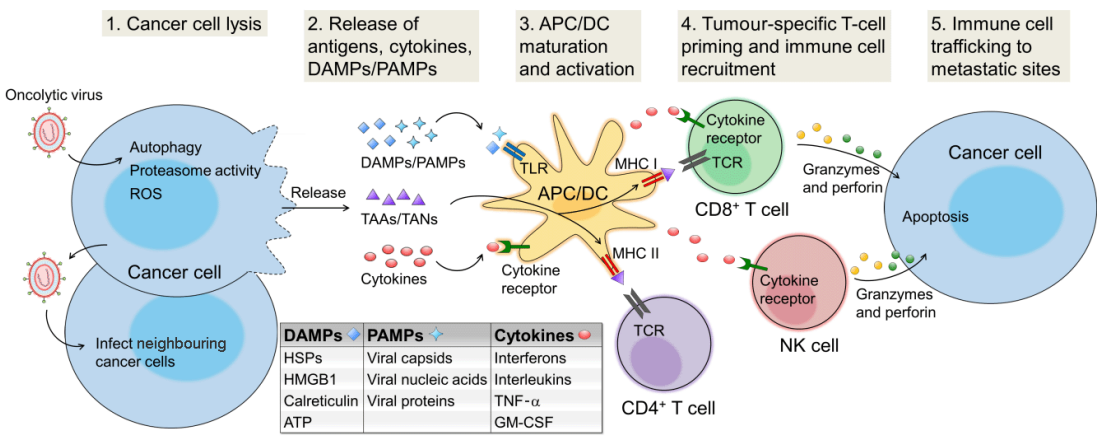

Figure 1. The generalised overview of OV-induced anti-tumour immunity. Initially, OVs infect primary cancer cells and cause direct oncolysis via inducing autophagy, increasing proteasome activity, and upregulating ROS caused by ER stress and genotoxic stress upon the infection. Subsequently, DAMPs and PAMPs trigger TLR, a major sub-family of the PRRs, and activate APCs. APC/DC uptake TAAs/TANs and express them to immune cells such as $CD8^+$ T cells and $CD4^+$ T cells through MHC I – TCR and MHC II – TCR interactions, respectively. The releasing cytokines and chemokines recruit both innate immune cells such as neutrophils, macrophages, NK cells, and DC, and adaptive immune cells such as T cells and B cells to the infected sites. In addition, APC helps stimulating and manipulating $CD8^+$ T cells and NK cells to release granzymes and perforin causing apoptosis of cancer cells. Furthermore, cytotoxic T lymphocytes can migrate to a distant tumour, recognize tumour antigens, and kill cancer cells.[9, 14, 46, 48, 59, 60] APC: antigen presenting cells; ATP: Adenosine triphosphate; DAMPs: damage-associated molecular patterns; DC: dendritic cells; ER: endoplasmic reticulum; GM-CSF: granulocyte macrophage colony-stimulating factor; HSP: heat shock protein; HMGB1: high mobility group protein; MHC: major histocompatibility complex; NK cell: natural killer cell; OV: oncolytic virus; PAMPs: pathogen-associated molecular patterns; PRR: pattern recognition receptor; ROS: reactive oxygen species; TAAs: tumour-associated antigens; TANs: tumour-associated neoantigens; TCR: T cell receptor; TLR: Toll-like receptor; TNF-α: tumour necrosis factor-α. Adapted from Kaufman et al.[14]

histocompatibility complex class I to $CD8^+$ T cells and $CD4^+$ T cells, respectively, leads to T cell priming and inflammatory responses.[60] The increase of proinflammatory cytokines such as IFN-1, interleukins (i.e. interleukin-1β, interleukin-6, interleukin-12), tumour necrosis factor-α, and granulocyte macrophage colony-stimulating factor, and the chemokines such as C-C motif chemokine ligand 2, C-C motif chemokine ligand 3, C-C motif chemokine ligand 5 and C-X-C motif chemokine 10 are beneficial for activating and recruiting both adaptive and innate immune cells against the primary tumour that is exposed to the virus and even in metastatic sites.[9, 46]

Different Strategies to Improve Oncolytic Viruses through Genetic Engineering

Manipulating viral genome has become the common strategy to apply OVs in cancer immunotherapy (**Table 2**). OVs can be genetically modified to enhance the anti-tumour immunity by employing immunostimulatory elements, and to improve the safety, efficacy, and targeted delivery of OVs.[13] Additionally, OVs can be genetically engineered to selectively target the unique receptors on the surface of cancer cells, providing greater safety for healthy cells.

Table 2. Summary of important genetic engineering in OVs

Genetic modification				
Gene deletion	**Gene insertion**	**Purpose/aim**	**Example of OVs**	**References**
ICP34.5		Selectively replicate in cancer cells, which have impaired PKR activity	Herpesvirus	61
ICP6	LacZ	Selectively replicate in cancer cells, which have sufficient level of host RR and $p16^{INK4A}$ tumour suppressor inactivation, avoid RR encoding	Herpesvirus	48, 61, 66
E1A		Restrict viral proliferation in healthy tissue	Adenovirus	61
g34.5		Restrict viral proliferation in healthy tissue and reduce neurovirulence	Herpesvirus	48, 62
a47		Increase anti-tumour immunity	Herpesvirus	63
TK		Selectively replicate in cancer cells	Vaccinia virus	68
	GM-CSF	Increase anti-tumour immunity	Herpesvirus, vaccinia virus, and adenovirus	58, 59, 64
	Endostatin	Destroy tumour vasculature and enhance therapeutic efficiency	Herpesvirus	13
	TSP1	Destroy tumour vasculature and enhance therapeutic efficiency	Herpesvirus	13
	GLAF-2	Increases anti-angiogenic and anti-tumour properties	Vaccinia virus	67

Note: GLAF-2: vascular endothelial growth factor-2; GM-CSF: granulocyte macrophage colony-stimulating factor; ICP: infected cell protein; OV: oncolytic virus; PKR: protein kinase R; RR: ribonucleotide reductase; TK: thymidine kinase; TSP1: thrombospondin-1.

For example, a deletion of ICP34.5 and ICP6 genes drives herpes simplex virus (HSV-1) to have replicative selectivity in tumour cells that show $p16^{INK4A}$ tumour suppressor inactivation, a common deficit in cancers.[61] A deletion of E1A gene, which functions to promote S-phase entry via retinoblastoma signalling pathway in adenovirus, can prevent viral replication in normal cells.[61] Furthermore, a deletion of γ34.5 gene in HSV-1 also renders the virus unable to replicate in normal cells because γ34.5 gene functions to impede a shutoff of host protein synthesis.[62] Anti-tumour immunity can also be enhanced by the deletion of viral genes such as α47 gene in HSV-1 which functions to antagonize the transporter associated with antigen presentation in host's cells.[63] The insertion of granulocyte macrophage colony-stimulating factor gene in HSV-1, vaccinia virus, and adenovirus can enhance the cytokine secretion for immune cell recruitment and stimulation.[58, 59, 64] Inserting the *Escherichia coli LacZ* gene into the ICP6 coding region in HSV-1 inactivates the ICP6 gene which encodes a viral ribonucleotide reductase function that allows viral DNA synthesis.[65] It promotes the selective viral replication within the cancer cells because the rapidly dividing cells express sufficient level of host ribonucleotide reductase.[66] To destroy tumour vasculature and enhance therapeutic efficiency, endostatin and thrombospondin-1 genes have been inserted in HSV-1 to suppress angiogenesis.[13] Vaccinia virus encoding a single-chain antibody against vascular endothelial growth factor-2 also increases anti-angiogenic and anti-tumour properties.[67] Furthermore, the deletion of the viral thymidine kinase (TK) gene in vaccinia virus increases the selectivity of OVs to cancer cells since tumour cells produce higher levels of TK.[68]

Viral Vector Systems Used for Oncolytic Therapy

Virus particles are broadly used in cancer treatment as gene delivery vehicles and as OVs.[69] The viral-based vectors for gene delivery can be used in targeted transfer of therapeutic genetic materials such as tumour suppressor genes, tumour-associated antigens, small interference RNA, pro-inflammatory factors, immune checkpoint inhibitors, and anti-angiogenic proteins.[70] Furthermore, OVs including many virus families are often additionally armed in order to enhance therapeutic efficiency and induce an anti-cancer immune response.[71] In clinical trials for cancer treatment, retrovirus (including lentivirus), adenovirus, and poxvirus vectors are commonly used.[70] Other virus vectors such as HSV, adeno-associated virus, measles virus, VSV, and poliovirus can also be found in the clinical trials.[72]

Clinical Trials and Applications of Oncolytic Viruses

The novel medical approaches including oncolytic cancer therapy inevitably need efficient tests to analyze their ability before being widely used or executed in the populations. Normally, clinical trials of various OVs would examine the safety, toxicity, efficacy, adverse effects, maximum tolerated dose, biomarkers, anti-tumour mechanism, and immune responses. These experiments could confine the effective and safe modified viruses to be used in cancer patients with fewer off-target delivery, lower rate of normal cell lysis, and less severe side effects. In addition, we could acknowledge the practical doses for patients and the mechanism of drugs towards cancer cells and the immune system against OV therapy. Here we summarize the clinical trials of OVs in different phases with various OVs in recent years. They would show the early stage of clinical proceedings, and the efficacy of the virus across many cancer types. The clinical trials included in this review were collected from published years between 2015–2023. The information of the recent clinical trials phases I,[73-88] II,[102,103] and III[89-101] are presented in **Tables 3–5**, respectively.

Various Approaches to Improve Oncolytic Virus-Delivery Specificity

Recently, viral delivery has played a vital role and extensively obtained attention in preclinical and clinical trials of biomedical fields. Owing to the specific and special properties of viruses, including great biocompatibility and biodegradation in cellular environments and minimal toxicity, viruses have been utilised as biological carriers of drugs, genes, and active chemical compounds.[104, 105] Therefore, the viral delivery platform is nowadays developed and evolved in many therapeutic applications, such as vaccines, gene therapies, immunotherapies, cancer therapies, anti-microbial therapies, cardiovascular therapies, imaging, and theranostics. Particularly, OV delivery offers efficient anti-tumour treatment and immunovirotherapies with potential advantages of high safety, specificity, selectivity, and efficacy.[20, 62, 106] On the other hand, designing the oncolytic viral delivery system is still a huge challenge and under examination due to the restrictions of bioavailability. The systematic administration of OVs usually can trigger strong host immune responses, resulting in the virus inactivation and clearance as well as weakened therapeutic anti-tumour effects by the production of neutralizing antibodies in bloodstream.[107, 108] In order to address these issues, several of the following approaches were introduced.

OVs encapsulation approaches

The encapsulation of OVs exhibits a novel alternative strategy for facilitating cellular administration against the immune system.[109] Numerous biomedical research works have been progressively reported over the past couple of years corresponding to the OVs encapsulation approaches for cancer treatment.

Bioactive polymers

Virus encapsulation using bioactive polymers and nanoparticles is a fascinating alternative to enhance protection from host immune system and improve anti-cancer therapeutic performance. [110-112] In 2021, Garofalo et al.[113] designed a viral delivery platform by coating polygalactosyl-b-agmatyl (Gal_{32}-*b*-Agm_{29}) diblock copolymer with asialoglycoprotein receptor on adenovirus Ad5/3-D24-ICOSL as OVs towards the effective hepatocellular carcinoma treatment in liver cells, as illustrated in **Figure 2**. They revealed that the polymer coated-OVs system potentially showed a significant improvement in the infectivity, viral replication, the lysis of tumour cells, and immunogenic cell death together with high safety and efficacious therapeutic effect. However, some studies revealed that synthetic polymers and nanoparticles as carriers provided unpreferred delivery efficiency and elevated toxicity triggering undesirable side responses in host surroundings and improved tumour proliferation.[114-116] The alternative designs of polymeric materials for virus delivery should focus on the biocompatible polymers and OVs' capture-release efficiency. The natural polymers or naturally derived polymers linking through dynamic covalent bonds could be utilised to deplete the mentioned downsides.

Liposome and extracellular vehicles

Lipid-based non-viral encapsulation is recognised as an attractive systematic viral delivery strategy for OVs. Liposome and extracellular vehicles containing phospholipid bilayer membranes act as protective carriers to eliminate the limited effects of immune clearance and increase cellular uptake into target cells.[117-119] In 2019, Wang and coworkers[120] utilised oncolytic alphavirus M1, an anti-tumour infector, encapsulated into liposome (M-LPO) to kill and infect zinc finger anti-viral protein-deficient tumour cells in LoVo and Hep 3B cell lines, as seen in **Figure 3**.[121] The M-LPO delivery system illustrated a well-defined cellular administration with weakened intrinsic M1 immunogenicities and the attenuated responses of neutralizing-antibody production for improving anti-cancer therapies. Besides, in the next three years, Huang and his colleagues[122] synthesised the novel viral delivery platform using cationic 1,2-dioleoyl-3-trimethylammonium-propane-folate liposomes (Df) encapsulating oncolytic-competent adenovirus (TAV255-Df) for the gene and anti-tumour therapies of the CAR-deficient tumours, such as CT26 colon carcinoma murine cells, as depicted in **Figure 4**. The TAV255-Df liposome-encapsulated platform could efficiently circumvent the requirement of coxsackievirus and CAR for cell entry and express the increment of viral transfection, tumour regression, and prolonged survival rate. However, occasionally some unencapsulated OVs could be observed as they were not loaded inside liposomes and remained in suspension. Thus, the purification of OVs from the liposome-encapsulated OVs is still a challenge for this delivery platform.[123, 124]

Nanohydrogels

As a nano-scale delivery system, nanohydrogels combine the advantages of the hydrogel system and nanoparticles, including tissue-like mechanical properties, great biocompatibility and biodegradability, potential surface reactivity, and probability for efficient cellular entry.[125, 126] Hence, nanohydrogels have been considered as an attractive candidate for protective OVs

Table 3. Summary of major oncolytic virus recently under clinical trials: phase I study

Virus type	Name (published year)	Cancer	Location	Patients number	Measurement	Route of administration	Study conclusion
Herpes virus	G207[73] (2022)	Glioblastoma	USA	6	Correlated gene analysis	Intratumoural stereotactic injection	The results have shown approximately 500 tumour-associated genes expression correlating to the patient's survival rate. The enhancement of T-cell and IFN production also affected the immune system. In the longest survival patient, there was the highest T-cell-related gene expression.
	OrienX010[74] (2022)	Melanoma	China	26	Safety, tolerability, efficacy, and phase II dose level	Intratumoural injection	The oncolytic OrienX010 was safe and well-tolerated in patients with melanoma. This therapeutic method exhibited significant anti-tumour activity. The recommended dose for phase II clinical trials without severe AEs was 10 mL of 8×10^7 pfu/mL every 2 weeks.
Adenovirus	ICOVIR-5[75] (2019)	Cutaneous and uveal melanoma	Spain	12	Toxicity and efficacy	Intravenous injection	Tumour targeting is possible but more efficient tumour debulking is needed via oncolysis due to the immune system leading to low anti-tumour efficacy. The toxicity was very short without inflammatory response syndrome.
	DNX-2401[76] (2018)	Malignant glioma	USA	25	Safety, efficacy, and biologic effects	Intravenous injection	25% of patients with single DNX-2401 survived more than 3 years and 95% decrease in tumour volumes was observed in three patients. This is due to oncolytic effects and emerging immune-mediated anti-glioma response
Coxsackie virus	CVA21[77] (2019)	AML	UK	16	Anti-tumour ability and cellular mechanism responsible	Intravenous delivery	CVA21 can activate the immune system for anti-tumour activity comprising cytokine-mediated bystander killing, enhancing of natural killer cell-mediated cellular cytotoxicity and tumour-specific cytotoxic T lymphocytes. Type I IFN and NK cell activation was observed. Moreover, the crucial mediators are ICAM-1 and plasmacytoid dendritic cells.
	CVA21[78] (2019)	Non-muscle invasive bladder cancer (NMIBC)	UK	15	Safety, MTD, evidence of viral replication, induction of inflammatory cytokines, anti-tumour activity, and viral-induced changes in resected tissue	Intravesical administration	All patients showed no sign of toxicity in both virus and virus with subtherapeutic dose mitomycin C. Inflammation of NMIBC tissues was observed with the increasing immune checkpoint inhibitory genes (PD-L1 and LAG3) and Th1-associated chemokines.
Measles virus	MV-NIS[79] (2017)	Myeloma	USA	32	MTD,	Intravenous delivery	The maximum tolerated dose of the patient with MV-NIS was not reached. Phase II with TCID50 1011 will be evaluated. MV-NIS is capable of replicating before being cleared by the immune system.
Poliovirus	PVSRIPO[80] (2021)	Melanoma	USA	12	Safety and tolerability	Intratumoural injection	The study showed well-tolerated with no SAEs or DLTs
	PVSRIPO[81] (2022)	Melanoma	USA	12	Immunologic effects in the TME	Intratumoural injection	Patients with lerapolturev and anti-PD-1 therapy have a median PFS of 2.3 years and had higher $CD8^+$ T cell infiltrates in prelerapolturev tumour biopsies.
Vaccinia virus	GL-ONC1[82] (2018)	Peritoneal cancer	Germany	9	Safety assessment, MTD, anti-tumour activity, viral replication, clinical efficacy, and biological effects in real-time study	intraperitoneal injection	GL-ONC1 administration into the peritoneal cavity was tolerated in advanced stage peritoneal carcinomatosis patients. There were limited efficient tumour cell infection, virus replication, and oncolysis.
	ACAM2000[83] (2019)	AML	USA	26	safety and feasibility	Intravenous, intratumoural, and intraperitoneal injections	ACAM2000 treatment delivered by autologous adipose SVF cells in AML patients was safe and well tolerated. Many patients showed great signals of anti-cancer effect.
	Olvi-Vec[84] (2021)	Platinum-resistant/ refractory ovarian cancer (PRROC)	USA	12	Safety, adverse events assessments,	intraperitoneal injection	Intraperitoneal Olvi-Vec oncolytic viral therapy illustrated well safety, clinical activities, and immune activation in PRROC patients.
Reovirus	PD-L1 with pelareorep and pembrolizumab[85] (2022)	PDAC	USA	11	Safety, DLT, tumour response, reovirus replication, and immune analysis	Intravenous injection	Chemotherapy of pelareorep and pembrolizumab showed no toxicity and provided great efficacy. The pelareorep and anti–PD-1 therapy evaluation was ongoing.
	Reovirus[86] (2020)	Metastatic CRC	USA	8	Immune response, cytokine expression pattern in peripheral circulation, exosomal and cellular microRNA levels, and effects of reovirus on leukocyte transcriptome	Intravenous infusion	Reovirus as an oncolytic agent provided multi-layered effects in tumour patients. Reovirus can function in immune stimulants, including immuno-chemo-therapeutic drugs and an oncolytic agent efficacy. Reovirus caused lysis of tumour cells, and facilitator of immune-mediated recognition.

Table 3. Continued

Virus type	Name (published year)	Cancer	Location	Patients number	Measurement	Route of administration	Study conclusion
Parvovirus	H-1PV[87] (2017)	Glioblastoma	Germany		Safety and tolerability, virus distribution, and MTD	Intratumoural or intravenous injection	H-1PV treatment was safe and well tolerated, and no reached MTD. The virus could cross the blood-brain/tumour barrier and spread through the tumour.
Seneca Valley virus	NTX-010 with cyclophosphamide[88] (2015)	Relapsed/refractory neuroblastoma, rhabdomyosarcoma, carcinoid tumour, and adrenocorticocarcinoma	USA	13	MTD and recommended phase II dose	Intravenous injection	NTX-010 is well tolerable at the dose levels in relapsed/refractory solid tumours pediatric patients. The addition of cyclophosphamide showed limited applicability.

Note: AE: adverse event; AML: acute myeloid leukemia; CRC: colorectal cancer; DLT: dose-limiting toxicity; ICAM-1: intercellular adhesion molecule 1; IFN: type I interferon; MTD: maximum tolerated dose; NK cell: natural killer cell; ORR: response rate; PDAC: pancreatic ductal adenocarcinoma; PFS: progression-free survival; SAE: serious adverse event; SVF: stromal vascular fraction; TCID50: 50% tissue culture infectious dose; Th: T helper; TME: tumour microenvironment.

Table 4. Summary of major oncolytic virus recently under clinical trials: phase II study

Virus type	Name (published year)	Cancer	Location	Patient number	Measurement	Route of administration	Study conclusion
Herpes virus	OH2[89] (2021)	Various	China	40	Safety and tolerability	Intratumoural injection	This phase I/II study showed that the oncolytic virus OH2 was safe and well-tolerated in patients with solid tumours. The durability of anti-tumour activity was significantly remarkable in patients with metastatic esophageal and rectal cancer.
	G47Δ[90,91] (2022)	Glioblastoma	Japan	13	Safety and tumour response	Intratumoural injection	Study showed safety of G47Δ up to 1×10^9 pfu/dose for two doses within 14 days. It could cause immediate infiltration of lymphocytes that directed towards tumour cells. Three of 13 patients had long-term survival (> 46 months) from the delayed effect of anti-tumour immunity.
				19	Efficacy	Intratumoural injection	The study showed the overall response in 2 years, partial response in 1 patient and stable disease in 18 patients. The number of tumour-infiltrating $CD4^+/CD8^+$ lymphocytes and persistent low numbers of $Foxp3^+$ cells increased which was evidenced by biopsies. It also showed that G47Δ was safe for oncolytic cancer therapy.
Herpes virus	T-VEC[92,93] (2021)	Breast cancer	USA	35	Efficacy, overall response rate ORR, rates of local overall response/disease, control rate, PFS, and OS	Intratumoural injection	In patients with inoperable locoregional recurrence of breast cancer, intratumoural T-VEC as monotherapy was not therapeutically desirable owing to uncontrolled disease progression.
		STS	USA	30	Safety, tolerability, and efficacy	Intratumoural injection	The incorporation of TVEC and EBRT provided safety and good tolerability towards STS treatment. These can also increase the immune response without necrosis. The result also evidenced that Caspase-3 could be a biomarker relating to a positive effect of TVEC.
Adenovirus	CG0070[94] (2017)	NMIBC	USA	45	Safety and efficacy in patients with high-risk BCG-unresponsive NMIBC	Intravesical injection	The toxicity of virotherapy was relatively low. There was 47% CR of patients with high-risk BCG-unresponsive NMIBC, 58% CR of patients with CIS, and 50% of patients with CIS-containing tumours.
Coxsackie virus	V937[95] (2021)	Melanoma	USA	57	Efficacy and safety in patients with unresectable stage IIIC or IV melanoma	Intratumoural injection	V937 was well tolerated and warrants further investigation for treatment of patients with unresectable melanoma without additional toxicities. The primary efficacy endpoint was 38.6% and durable response rate was 21.1%. 12-month PFS was 32.9% and 12-month OS was 75.4%
Vaccinia virus	JX-594[96] (2022)	Soft-tissue sarcoma	France	20	The 6-month non-progression rate, efficacy, immune response, and therapeutic potential	Intravenous injection	The administration of JX-594 oncolytic virus was safe in advanced STS patients. The role of immune-oncology agent combination and the patient population identification who received benefit from this approach were questions from major interest.

Table 4. Continued

Virus type	Name (published year)	Cancer	Location	Patient number	Measurement	Route of administration	Study conclusion
Reovirus	FOLFOX/BEV with pelareorep[97] (2018)	Metastatic colorectal cancer	Canada	103	PFS, OS, ORR, and correlative analyses.	Intravenous injection	FOLFOX/BEV with pelareorep was increased ORR, but PFS was reduced. Reduction of treatment intensity with standard agents provided the lack of pelareorep benefit.
	Pelareorep (reolysin) with pemetrexed or docetaxel[98] (2018)	NSCLC	Canada	166	PFS, OS, ORR, and exploratory translational analyses.	Intravenous injection	No improvement of PFS in NSCLC patients was demonstrated in pelareorep chemotherapy.
	Paclitaxel/ Pelareorep[99] (2018)	mBC	Canada	81	PFS, response rate, OS, circulating tumour cell counts, safety, and exploratory correlative analyses	Intravenous injection	This randomised phase II study of pelareorep and paclitaxel was not different in PFS for treated mBC patients. Pelareorep/paclitaxel combination revealed longer OS.
Parvovirus	H-1PV (ParvOryx)[100] (2021)	metastatic PDAC	Germany	7	Safety, clinical efficacy, virus pharmacokinetics, shedding, and immune response	Intravenous injection	No environmental risks were indicated immune modulation once ParvOryx injection. H-1PV was systematic clinical development with immunomodulatory compounds.
Seneca Valley virus	NTX-010[101] (2019)	ES SCLC	USA	50	PFS, prespecified interim analysis for futility, viral clearance, and the development of neutralizing antibodies	Intravenous injection	NTX-010 treatment had no benefit with ES SCLC patients. Persistence of NTX-010 was related a short PFS. There was no outcome improvement of NTX010 treatment in ES SCLC patients after platinum-based chemotherapy.

Note: BCG: Bacillus Calmette-Guerin; CR: complete response; EBRT: external beam radiation therapy; ES SCLC: extensive-stage small cell lung cancer; mBC: metastatic breast cancer; NMIBC: non-muscle invasive bladder cancer; NSCLC: non-small cell lung cancer; ORR: objective response rate; OS: overall survival; PDAC: pancreatic ductal adenocarcinoma; PFS: progression-free survival; STS: soft-tissue sarcoma.

Table 5. Summary of major oncolytic virus recently under clinical trials: phase III study

Virus type	Name (published year)	Cancer	Location	Patients number	Measurement	Route of administration	Study conclusion
Herpes virus	T-VEC[102] (2017)	Melanoma	UK	437	Efficacy	Intratumoural delivery	Patients with early metastatic melanoma (stage IIIB–IVM1a) had a high CR rate and durability with T-VEC administration. The results still showed the well-tolerated ability of T-VEC and also exposed the association between the virus and the survival rate.
	T-VEC[103] (2019)	Melanoma	USA	41	Safety	Intralesional injection	The results showed consistent safety as previous research of T-VEC. Only influenza-like symptoms were observed which are mild or moderate AEs

Note: AE: adverse event; CR: complete response.

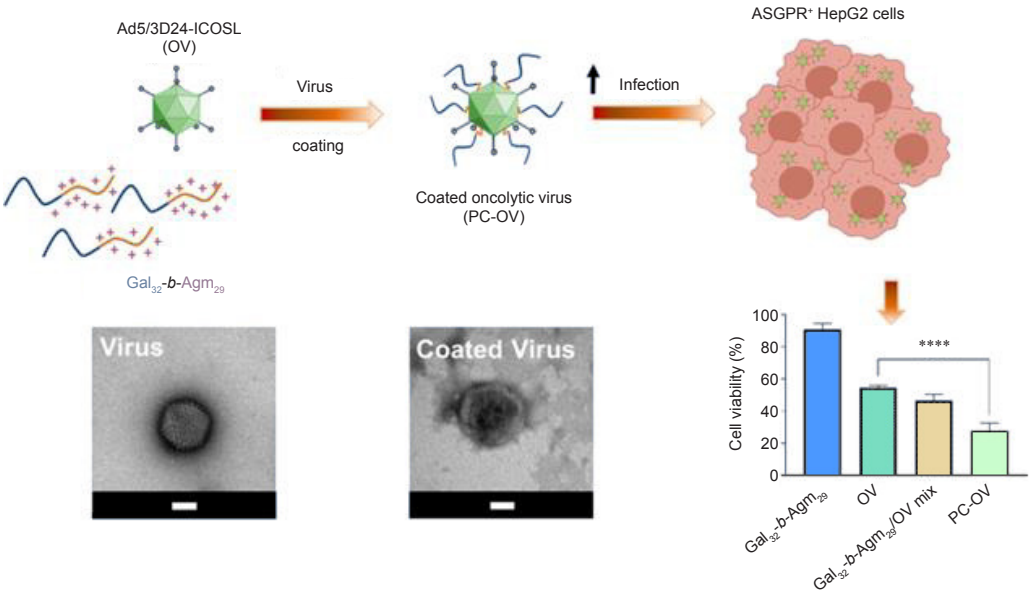

Figure 2. The design of a high potential therapeutic polymer-coated oncolytic viruses (PC-OVs) delivery system which is coated by electrostatic polygalactosyl-b-agmatyl (Gal_{32}-*b*-Agm_{29}) diblock copolymer with asialoglycoprotein receptor (ASGPR) for diagnosis of hepatocellular carcinoma in human hepatoma cell line HepG2 as a model. ****$P < 0.0001$. Reprinted from Garofalo et al.[113] OV: oncolytic virus; PC: polymer-coated.

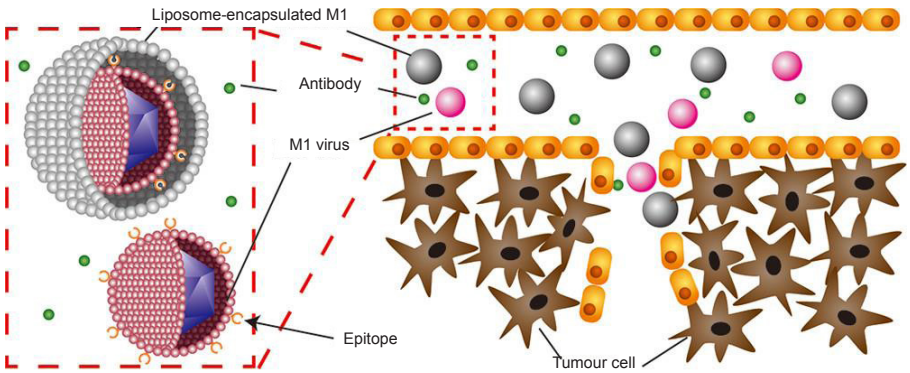

Figure 3. Schematic illustration of the fabrication of a liposome-encapsulated M1 virus platform (M-LPO) for tumour therapy in LoVo and Hep 3B cell lines. Reprinted with permission from Wang et al.[120] Copyright 2019 American Chemical Society.

delivery. In 2021, Deng and coworkers[127] developed an OV delivery system employing a prostate-specific adenovirus [I/PPT-E1A] (DNA virus) and echovirus Rigvir® ECHO-7 (RNA virus)-encapsulated hyaluronic acid-based redox responsive nanohydrogels for anti-tumour therapies (**Figure 5**). The OVs-loaded nanohydrogel platform could promote systematic cellular administration and viral releasability equipped with redox stimulation in cancer cells with highly promising safety and efficacy. Furthermore, this platform illustrated the limited anti-virus neutralizing antibody and reduced immune response.

Carrier cell-based delivery approaches

Carrier cell-based delivery is a process of using a suitable cell as a viral carrier to facilitate the ferrying. These cells should protect the therapeutic substances from inevitable biodegradation, increase the density of viral agglomeration at tumour cells and reduce the side effects of cancer therapy. Stem cells have been broadly used as carriers for numerous therapeutic agents as well as OVs because these carriers would not be filtered by the liver and neutralised via the immune system. Therefore, they could directly access the tumour cells and release the curing viruses after their replication. In this section, the strategies for utilizing mesenchymal stem cells (MSCs), neural stem cells (NSCs), menstrual blood-derived stem cells, tumour-infiltrating lymphocytes, and cryo-shocked cancer cells as carriers for the OVs will be discussed.

Mesenchymal stem cells

MSCs are multipotent stem cells that can be found in various sources in the body such as adipose tissue, umbilical cord, placental tissue, and bone marrow. MSCs have the ability to differentiate and express tumour-associated chemokines, which can benefit tumour targeting and accumulation. Moreover,

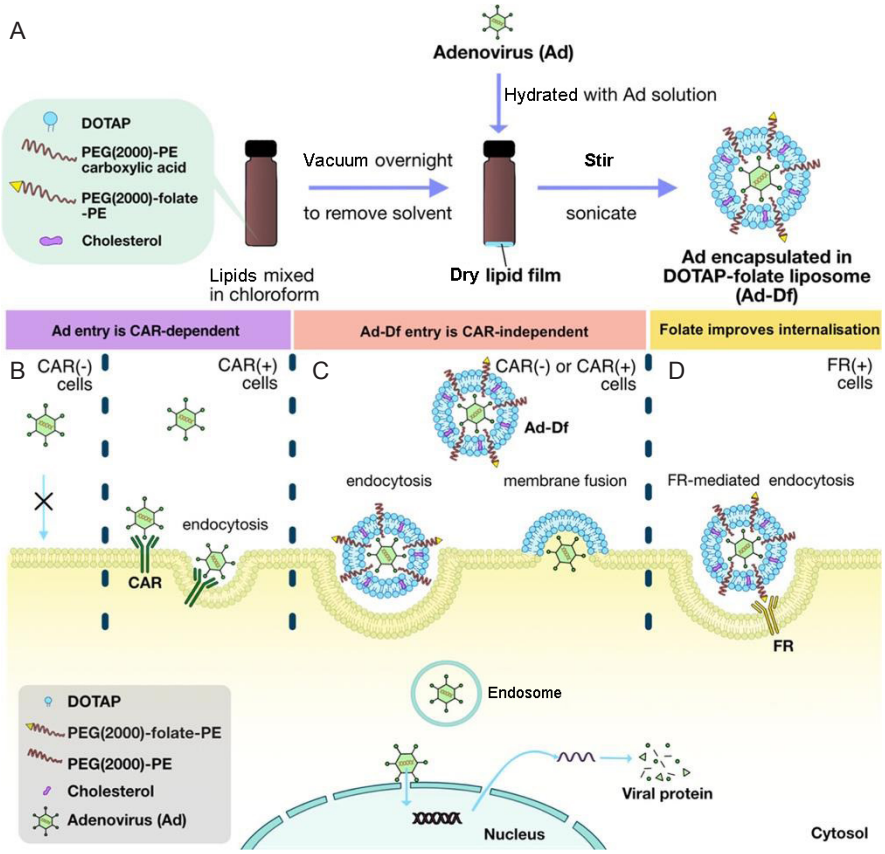

Figure 4. (A) The synthesis of adenovirus encapsulating cationic 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)-folate liposomes (Ad-Df). (B) The cellular penetration approaches of Ad-Df viral platform in various coxsackievirus and adenovirus receptor (CAR)-deficient cell lines. (C) Ad-Df is capable of entering the cells via endocytosis during no expression of CAR of the target cells, leading to transfect CAR-positive and -negative cells. (D) The cellular uptake into FR-positive cells can be enhanced by Ad-Df containing folate-conjugated lipid through FR-mediated endocytosis. Reprinted with permission from Huang et al.[122] Copyright 2022 American Chemical Society. FR: Folate receptor; PEG(2000)-PE: 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(poly-ethylene glycol)-2000].

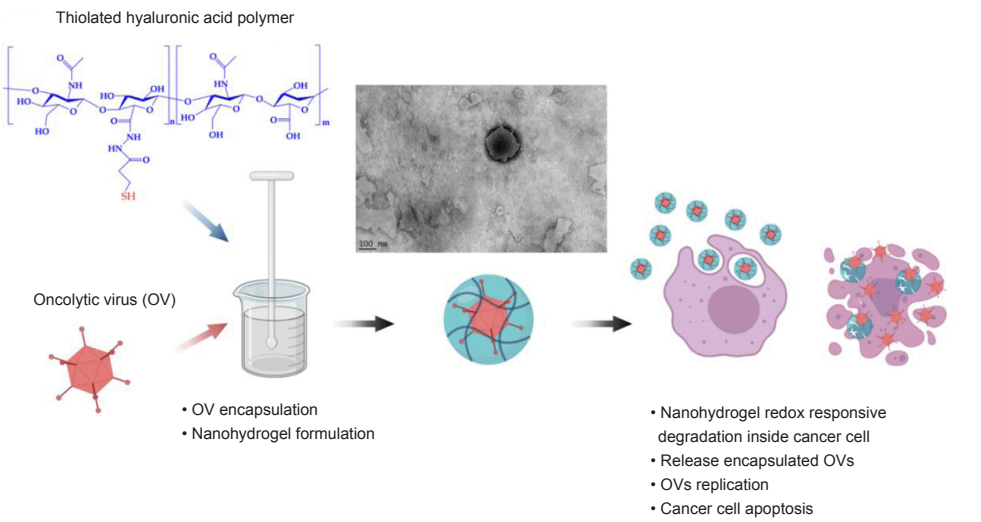

Figure 5. A hyaluronic acid-based nanohydrogel formulation for oncolytic viral delivery for anti-cancer therapy. Reprinted from Deng et al.[127]

MSCs can exhibit the tolerogenic microenvironment through the T-cell unresponsiveness or apoptosis induction and suppress the activity of natural killers, $CD8^+$ and $CD4^+$ cells, by releasing prostaglandins and interleukins) in blood. Therefore, MSCs could be employed as a carrier for OV delivery, as depicted in **Figure 6**.[128]

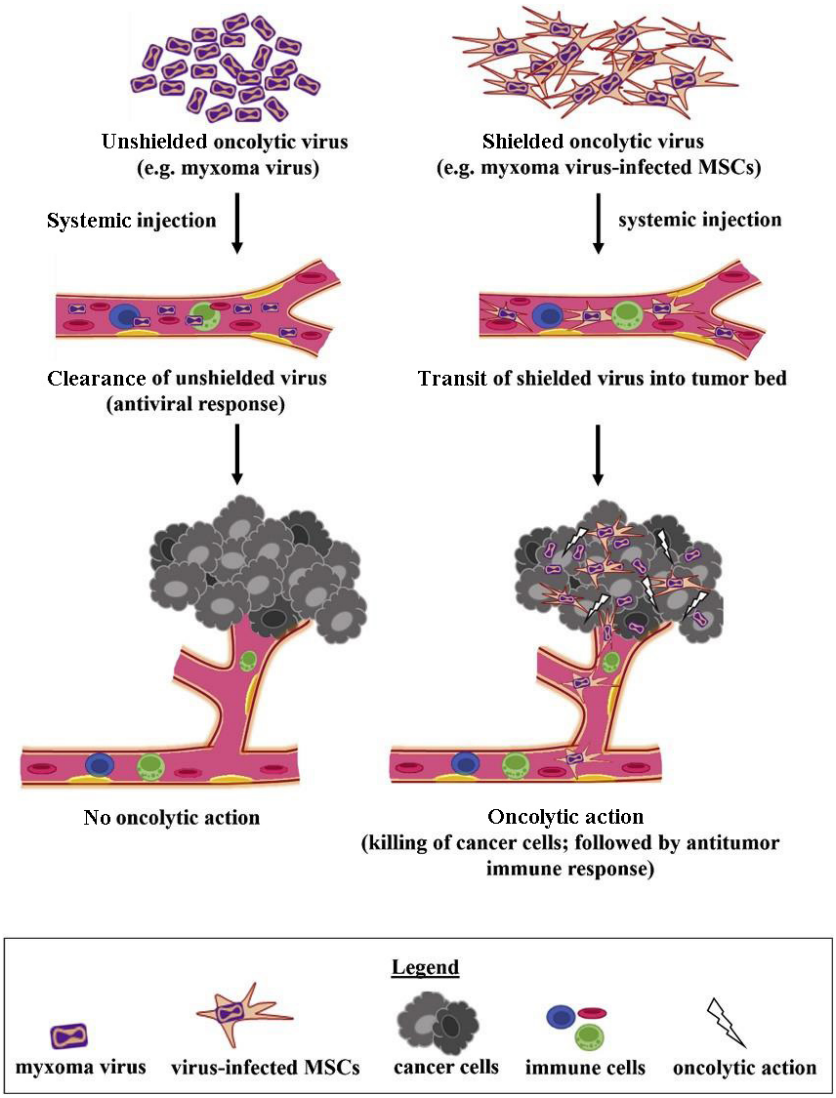

Figure 6. Application of myxoma virus-loaded mesenchymal stem cells (MSCs) for pulmonary melanoma treatment. Reprinted from Hadryś et al.[128]

Interleukin-15 derived-oncolytic myxoma cells carrying myxoma virus were delivered through intravenous administration via the bone marrow-derived MSCs as a viral carrier in order to treat pulmonary melanoma in mice.[129] The results of bioluminescence imaging showed the high tumour infectivity of the delivery process and enhanced oncolytic activity due to the immune response compared to blood and lungs. The levels of $CD8^+$ and $CD4^+$ in blood did not significantly increase at the endpoint of the experiment on the 21st day whereas those percentages were higher in the lung. In addition, the survival rate tended to be longer in mice that were injected with two doses of MSC-shielded carrying myxoma virus in comparison to MSC and unshielded carrying myxoma virus.

Neural stem cells

Malignant glioma, a brain and/or spinal cord cancer, is another severe disease that is rarely curable these days due to the rapid growth rate of the tumour cells and the prevention of chemotherapeutic agents by the blood-brain barrier. NSCs can serve as an effective vehicle for oncolytic therapy as they could penetrate blood-brain barrier, migrate to the tumour site, and distribute among the glioma cells. One recent study reported that the engineered oncolytic adenovirus, CRAd-S-pk7, delivered by NSCs had a longer survival rate of 50% in mice compared to the virus alone.[130] The clinical trial phase I treatment of NSC-CRAd-S-pk7 injected through the walls of the resection cavity affirmed the safety and feasibility of glioma therapy without formal dose-limiting toxicity.

In another study, oncolytic chimeric orthopoxvirus was chosen as a candidate for ovarian cancer treatment. CF33, a mutated chimeric orthopoxvirus in the J2R (TK) gene was delivered via the HB1.F3.CD21 clonal NSCs.[131] The precision of tumour targeting increased as the deficiency of TK in the CF33 could lead the NSCs loaded CF33 to the overexpressing TK area of tumour cells and thus, CF33 could infect, replicate, and kill the cancer cells. Another research showed the use of NSC.CRAd-S-pk7, NSCs that are transfected with glioma-tropic oncolytic adenovirus, to treat ovarian cancer (OVCAR8).[132] The number of tumours was reduced by more than 9-fold in 14 days in culture although the ratio of NSC.CRAd-S-pk7: OVCAR8 was 1:1000.

Menstrual blood derived MSCs

Menstrual blood contains MSCs which possess target tropism and low immunity. Menstrual blood therefore can be used to obtain menstrual blood derived MSCs, which are abundant and have a rapid growth profile. Guo et al.[133] reported that menstrual blood derived MSCs could be used to deliver engineered CRAd5/F11 chimeric oncolytic adenovirus, which could provide a large number of viruses within the colorectal cancer tumour area that efficiently expressed the tumour inhibitory activity within 7 days after injection. This could serve as a promising new source of stem cell carriers that successfully loaded and transferred the OVs to the desired targets.

Tumour-infiltrating lymphocytes/T cells

Tumour-infiltrating lymphocytes/T cell (TIL) therapy has been introduced to synergistically used as an anti-tumour agent with the oncolytic adenovirus.[134] The combination of those could enhance the treatment's ability to target by prevention of neutralization and kill the cancer cell. Oncolytic adenovirus coding for tumour necrosis factor-α and interleukin-2 (TILT-23) was intravenously and intratumourally delivered via TIL into tumours in mice. However, the relevant aspects of humans are still questionable and need further clarification.

Cryo-shocked cancer cells

Another state-of-the-art OV carrier is the cryo-shocked cancer cells in **Figure 7A**. The cancer cells were infected by an OV before treating with liquid nitrogen to eliminate the proliferation capacity and pathogenicity of the tumour cells.[135] After intratumoural injection as demonstrated in **Figure 7B**, the OV-loaded cancer cells could still release the targeting agent and have antitumour activity inside the tumour area. Moreover, the infiltration of $CD8^+$ was then increased by the activation of dendritic cells which could elevate the amount of anti-tumour cytokine and reduce the infiltration of regulatory T cells in tumours.

Conclusions and Perspectives

OVs utilise extensive mechanisms to preferentially infect and destroy cancer cells, including the specific replication in cancer cells causing direct oncolysis, the induction and stimulation of immunogenic cell death and anti-tumour immunity, and tumour vasculature which can be damaged by the infection of engineered OVs to tumour-associated vascular endothelial cells, leading to tumour necrosis and the infiltration of immune cells.[136] Genetically, engineered OVs have become the common promising strategy to enhance anti-tumour immunity, safety, and efficacy as well as targeted delivery, such as the deletion of ICP34.5, ICP6, E1A, γ34.5, α47, and TK gene, and the insertion of LacZ gene into the ICP6 coding region, and granulocyte macrophage colony-stimulating factor gene.

In the field of engineered OVs as anti-tumour agents, the major challenge for oncolytic virotherapy is the rapid viral clearance due to the host's immune response towards anti-viral immunity, as the primary results of the various physical and immunological defense mechanisms of the host cells, which are capable of virus inactivation and elimination. Manipulating the host immune system to minimize anti-viral responses and viral clearance, while immune-mediated tumour destruction is promoted, is a key to efficient oncolytic virotherapy. Hence, the design of systemic delivery carriers is of great significance and considerable effort has been made to obtain a novel and efficient platform for delivering OVs to targeted cells. An effective delivery method should minimize the viral clearance, reduce the off-target toxicity and enhance the tumour-specific responses. Numerous strategies have been developed to improve viral administration efficiency in preclinical and clinical settings, for instance, the encapsulation of OVs in polymeric scaffolds, nanoparticles, liposomes, extracellular vehicles, nanohydrogels, and carrier cells. The recent progress in genetic engineering and targeted delivery platforms will provide more opportunities for OVs with enhanced anti-tumour immunity, safety, and efficacy in cancer treatment and overcome clinical challenges.

The limitation of this review paper is that it provides information based on computerised or electronic databases. The hand-searching (manual process of screening) of journals, conference proceedings, and other publications was not included in the literature search process of this review.

Author contributions

Conceptualization and outline design of the paper: QW, MK; funding acquisition: QW, LC; manuscript review and editing: MK, TM, JJ, LC, QW. All authors approved the final version of this manuscript.

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Conflicts of interest statement

The authors declare no conflict of interest.

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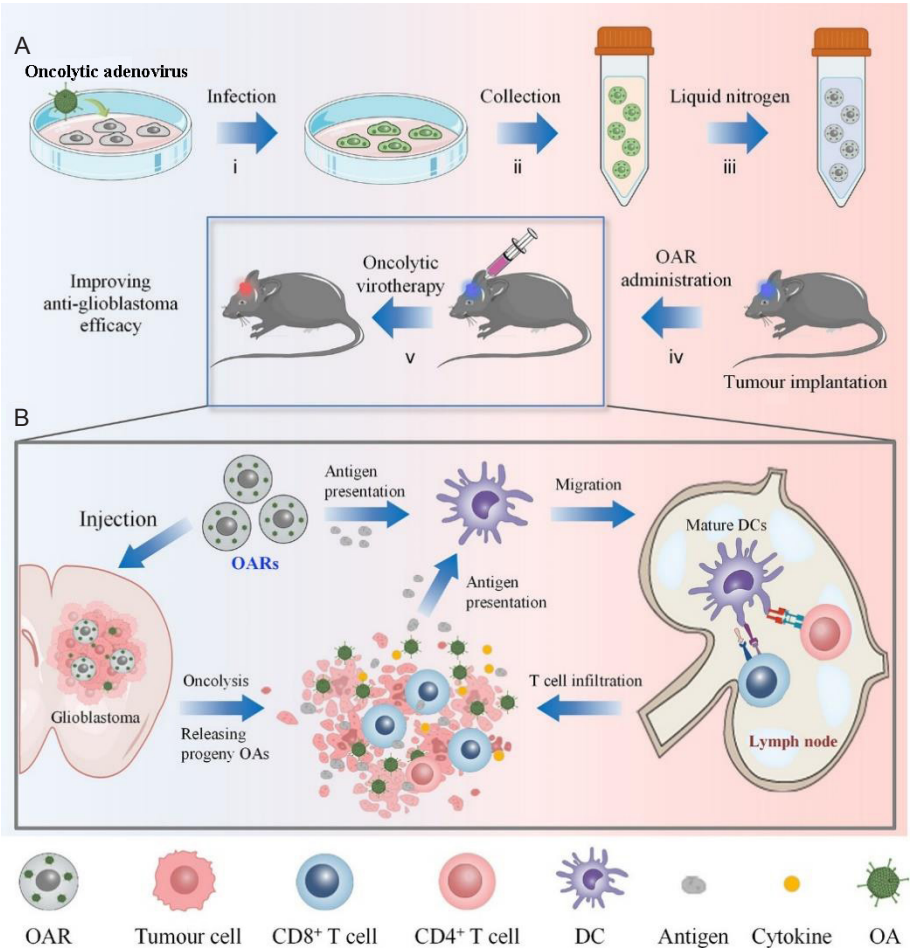

Figure 7. (A) Illustration of the preparation and *in vivo* experimental processes of cryo-shocked cancer cells as oncolytic adenovirus reservoir (OARs) for glioblastoma immunotherapy in a mouse glioblastoma model. (B) The administration mechanism of OARs via intratumoural injection. Reprinted with permission from Liu et al.[135] Copyright 2022 American Chemical Society. DC: dendritic cell; OA: oncolytic adenovirus.

Additional file

Additional file 1: Literature retrieval strategy.

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