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March 2026

Oncolytic Viruses: From Scientific Breakthrough to Manufacturing Reality

Abstract:

Oncolytic viruses (OV) represent a transformative class of cancer therapeutics designed to selectively infect, replicate within, and destroy malignant cells while stimulating systemic antitumor immunity. Advances in genetic engineering have further enhanced the antitumor potential of OV by enabling the delivery of therapeutic payloads, improving tumor cell selectivity, and even evading neutralization by the host immune system.

Unlike conventional cytotoxic therapies, OV combine direct tumor lysis, immune activation, and drug delivery, positioning them at the intersection of virology, immunotherapy, and gene therapy. This multimodal mechanism of action offers a compelling strategy for the treatment of refractory cancers.

Over the past decade, the field has transitioned from experimental concepts to clinical reality, with regulatory approvals in major markets and a robust pipeline of candidates in late-stage development. However, the successful translation of OV from bench to bedside depends not only on biological innovation, but also on the establishment of robust, scalable, cost-effective, and GMP-compliant (cGMP) manufacturing platforms.

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1. What are oncolytic viruses ?

Oncolytic viruses (OV) comprise a group of replication-competent viruses that are engineered or selected to preferentially infect and kill cancer cells while sparing normal tissues.

The concept of using viruses to treat cancer dates back to early clinical observations in the 20th century, when spontaneous tumor regressions were occasionally noted following natural viral infections. These observations led to several clinical trials attempting to treat cancers using wild-type or attenuated viruses[1].

Several factors drive tumor selectivity in oncolytic virotherapy, including the overexpression of cellular receptors involved in viral entry pathways, as well as defects in tumor suppressor genes or genes regulating cell cycle checkpoints, which favor OV infection and replication. Cancer cells also exhibit higher metabolic activity than normal cells, promoting the hijacking of the cellular genetic machinery for viral replication. Finally, cancer cells frequently display defects in antiviral innate immune responses, particularly in the type I interferon signaling pathway, which further contributes to increased viral permissiveness[2].

OV exert antitumor activity through two main and complementary mechanisms (**Figure 1**). First, following selective infection and replication within cancer cells, OV replication cycle induces cell lysis, leading to the release of progeny viruses. The production of newly formed OV within the tumor mass further amplifies tumor cell lysis. Second, cell lysis leads to the release of cytokines, tumor-associated antigens (TAAs), danger-associated molecular patterns (DAMPs), as well as pathogen-associated molecular patterns (PAMPs) derived from the OV itself. The detection of DAMPs and PAMPs by the innate immune system drives dendritic cell maturation, antigen presentation, and the activation of tumor-specific B- and T-cell immune responses. Through this modulation of the tumor microenvironment, OV can convert immunologically “cold” tumors into “hot” tumors, thereby enhancing both innate and adaptive antitumor immune responses.

In addition, the dissemination of OV within the host, together with the induction of a tumor-specific adaptive immune response, enables the elimination of distant metastatic cancer cells[3]. Furthermore, some OV-based therapies enhance tumor responses to radiotherapy and other immunotherapeutic modalities[4].

However, although OV preferentially target cancer cells, excessive oncolytic activity can cause off-target effects and increase toxicity, potentially damaging healthy tissues[5]. Moreover, some OV induce antiviral immune responses that result in their neutralization. These limitations can be addressed through combination approaches, including co-administration with low-dose chemotherapy, immunosuppressive cytokines[6,7] or delivery through nanomaterials[8]. These considerations should be addressed early in the development of OV-based therapies to ensure the safety of the final product and its clinical success.

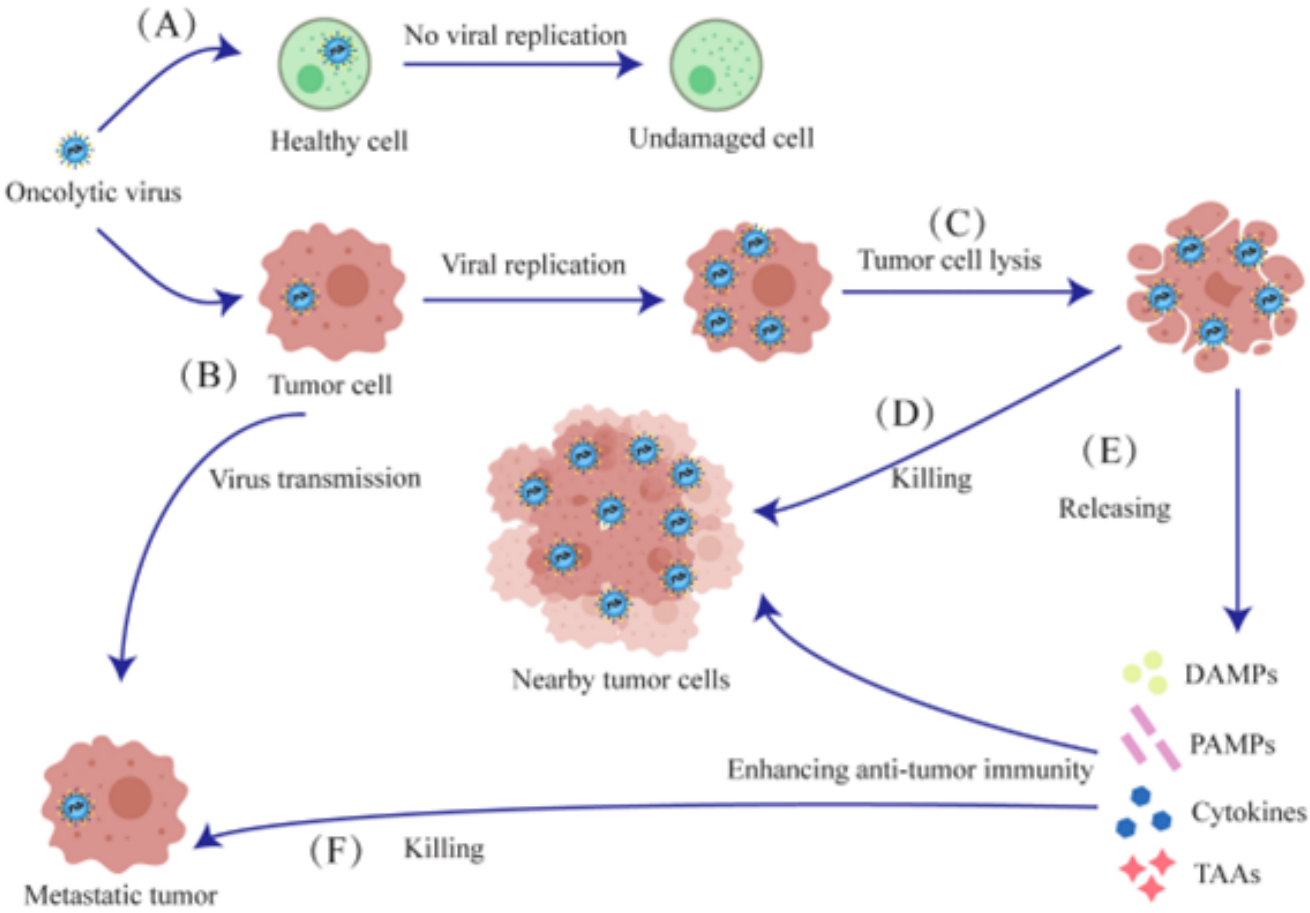


Figure 1. Antitumor activities of oncolytic viruses (OV). (A and B) OV selectively infect and replicate within cancer cells, (C) which ultimately lead to cell lysis. (D) The newly released OV then infect and lyse nearby and metastatic cancer cells. (E) Cancer cell lysis induces the release of cytokines, tumor-associated antigens (TAAs), danger-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs), which induce antitumor immunity against local and (F) metastatic tumors[3]

2. Development and diversity of oncolytic viral platforms

Early efforts in the 1960s and 1970s to treat cancer with naturally occurring viruses were largely discontinued due to the inability to adequately control viral pathogenicity. Interest in this approach later resurfaced with advances in genetic engineering, enabling the development of safer and more effective recombinant OV[9].

Recombinant OV are generated by incorporating transgenes into the viral genome. The use of recombinant OV can reduce viral immune clearance, enhance tumor tropism, limit pathogenicity, and boost antitumor activity by delivering therapeutic molecules directly within the tumor. Using OV as a delivery system protects the therapeutic cargo, overcomes limitations related to membrane permeability and poor diffusion, and addresses the reduced efficacy associated with short half-lives, as the therapeutic molecule is produced de novo with each viral replication cycle. OV also allow for more precise targeting of molecules within the tumor, thereby minimizing the risk of adverse toxicity[3,10,11].

A wide range of viral families has been studied for their potential in oncolytic virotherapy[12]. Most clinical studies have focused on DNA viruses, as their molecular biology and replication cycle are better understood. Their generally larger genomes also offer the advantage of facilitating recombinant gene expression[10]. On the other hand, non-enveloped viruses offer additional benefits, including reduced sensitivity to shear stress, which facilitates large-scale production processes, as well as greater physicochemical stability for formulation and storage, and improved biological stability once administered[13].

Adenoviruses, non-enveloped double-stranded DNA viruses, are among the most extensively studied OV and led to the approval of H101 (Oncorine) in China in 2005 for the treatment of head and neck cancers[14,15]. Their stable, episomal genome does not integrate into host DNA, allowing for safe deletions (e.g., E1A, E1B) and the insertion of transgenes. Adenoviruses exhibit strong lytic activity and can be produced at high titers. However, their broad tissue tropism may limit tumor selectivity and reduce efficacy in disseminated disease[12].

Herpes simplex viruses, enveloped double-stranded DNA viruses, are also extensively studied, as they offer several advantages, including a large genome that is easily manipulated, high lytic potential, and strong immunogenicity, although their natural neurotropism requires attenuation[16]. Talimogene laherparepvec (T-VEC), a herpes simplex virus type 1–based OV, remains the only OV widely approved by both the FDA and the EMA. T-VEC is indicated for the treatment of patients with recurrent melanoma after initial surgery and was first approved in 2015. It was engineered through the deletion of virulence genes such as ICP34.5 and ICP47 to enhance safety, while the insertion of the GM-CSF gene improves antitumor immune responses[10]. More recently, Teserpaturev, another herpes simplex virus type 1–based OV, was approved in Japan in 2021 for the treatment of patients with malignant gliomas[17].

Other viral platforms represent promising candidates for OV-based therapies, each offering unique properties and advantages. Among them, vaccinia virus, a large and complex enveloped double-stranded DNA virus, is characterized by rapid replication kinetics, including in acidic and hypoxic environments, which are characteristic of many solid tumor microenvironments. Its large genome also enables extensive genetic modification. Oncolytic vaccinia virus has the ability to infect a broad range of cancer cells, as it is highly dependent on the thymidine kinase gene, which encodes an essential enzyme for viral replication and is frequently overexpressed in cancer cells, but rarely in normal tissues. However, vaccinia-based OV are rapidly neutralized by the host immune system[18,19]. In addition, their large size (approximately 300 nm,[20]) prevents sterilization by 0.2 µm filtration, thereby requiring manufacturing under strict Grade B cleanroom conditions.

Reovirus, a non-enveloped double-stranded RNA virus, preferentially replicates in cells with activated Ras signaling pathways, which are common in many cancers. Reoviruses are generally

well tolerated and can be administered systemically, representing a significant advantage for the treatment of metastatic cancers. However, pre-existing anti-reoviral immunity in the general population, their moderate efficacy as monotherapy, and their limited capacity for genetic engineering constrain the clinical effectiveness of oncolytic reoviruses compared with other viral platforms[21].

Measles virus, vesicular stomatitis virus, and Newcastle disease virus, all enveloped single-stranded negative-sense RNA viruses, have demonstrated promising preclinical and clinical activity, particularly in hematological and solid malignancies. However, they are less extensively developed than large DNA OV due to their limited genetic cargo capacity, lower physicochemical stability inherent to their enveloped structure, and significant translational challenges related to antiviral immunity and safety[12].

Overall, the choice of an OV depends on tumor type and microenvironment, desired immunostimulatory effects, and the balance between safety, genetic flexibility, and lytic potency. The field is increasingly moving beyond simple oncolysis toward «armed» viruses, which serve as platforms for delivering therapeutic payloads such as cytokines, single-chain variable fragment antibodies (scFv), or bispecific T-cell engagers (BiTEs)[12,16].

3. Bioproduction challenges of oncolytic viruses

Although clinical progresses are promising, manufacturing remains a major bottleneck in OV development. Unlike non-replicative viral vectors, OV must retain infectivity and replicative capacity while meeting stringent safety and regulatory requirements.
The development of a robust and cGMP-scalable bioproduction process starts with the identification, generation, and characterization of starting biological materials, including cGMP cell banks and viral seeds.
Considering the producing cell line, beyond high permissivity to viral infection and replication, it should ideally exhibit high proliferation capabilities, the ability to grow at high density in suspension, and in serum-free culture. In this context, several cell lines are extensively used in the pharmaceutical industry for viral production and are already approved by regulatory authorities, including HEK and derivatives, Vero, MDCK, A549, BHK-21, WI-38, MRC-5, CV-1, DF-1, EB66, and PER.C6 cell lines[22–24]].

The bioproduction pipeline then continues with the upstream process, starting with cell amplification up to production scale. Depending on the cell type, amplification is carried out in static vessels (e.g. T-flasks and multi-layer vessels, roller bottles) or stirred containers (e.g. Erlenmeyer or spinner flasks), to ideally and ultimately reach production scale in stirred-tank bioreactors. The use of stirred-tank bioreactors is a gold standard in the biopharmaceutical industry, allowing high-density cell culture and ensuring homogeneous distribution of gases and nutrients in a closed system, fully controlled and regulated (temperature, pH, oxygenation, etc.). Their geometric and hydrodynamic characteristics are well described and characterized, facilitating scale-up to several cubic meters[25]. For anchorage-dependent cells, including Vero, MDCK, MRC-5, PER.C6, and others, microcarrier-based cultures are the preferred approach to enable suspension growth in stirred-tank bioreactors, while preserving their phenotype and functional properties[26].

Cell culture medium composition is also a major concern, notably the use of serum or animal-derived components, which raises safety concerns, reduces process standardization, and complicates final product purification. The selection or development of a serum-free, chemically

defined medium is therefore considered the ideal solution for achieving robust bioprocesses[27].

Once the culture has reached the targeted volume and cell density, viral infection is performed by inoculating the viral seed. Major parameters to consider include the multiplicity of infection (MOI), temperature, agitation and gas transfer conditions depending on the cells and viral hydrodynamic sensitivity (particularly for microcarrier-based cultures and enveloped viruses), as well as viral replication kinetics and stability to determine the optimal harvest time[28–31].

In addition, OV being cytopathic, the culture is intrinsically self-limiting. However, processes can be intensified through culture medium renewal[29,32], or by implementing fed-batch[33] or perfusion operation modes[34,35], and performing multiple harvests[36,37]. Continuous processes can also be implemented through feeding strategies involving fresh uninfected cells to sustain viral propagation. However, this may lead to the production of defective interfering particles and therefore requires careful attention[38].

Once the targeted viral production is achieved, the harvest is performed. A critical aspect during harvest is the intracellular viral load, which may necessitate a cell lysis step to maximize viral recovery. For example, viruses such as herpes simplex virus and vaccinia virus are largely intracellular, requiring mechanical, chemical, hypotonic, or freeze–thaw lysis methods to release the maximum number of viral particles prior to downstream processing[39–42]. However, lysis can impact viral integrity and increase host cell–derived impurities, notably DNA. This can be addressed by nuclease treatment, which degrades DNA into small fragments that will be removed during downstream processing[22,43].

A classical and scalable downstream process then starts with a first clarification step to remove large impurities, including cells and microcarriers, cell debris, some aggregates, and larger macromolecules through depth filtration or microfiltration[44]. The clarified harvest is then usually diafiltered and concentrated using tangential flow filtration devices. Depending on the virus of interest and product impurities, different cut-offs are available, enabling the removal of small impurities while the virus recirculates within the system[22].

The concentrated and pre-purified product can then be further purified using liquid chromatography technologies such as anion-exchange, affinity or size-exclusion. This step enables the binding and isolation of the virus of interest, while the remaining impurities are eliminated[39,45,46]. A diafiltration step is often necessary for final product polishing in order to remove chromatography buffer components[39,47].

Finally, sterilization is performed by 0.2 µm filtration for most OV, with the exception of larger viruses such as Vaccinia virus[20]. The final OV product is then formulated in an appropriate buffer ensuring its stability for short- to long-term storage below −20°C[45]. Some lyophilized formulations also enable storage at 4 °C, facilitating the supply chain while maintaining viral infectivity[48].

Thus, the definition of the downstream process as well as the formulation must balance purity, yield, and viral integrity, as excessive processing can compromise biological potency[46,49]. Notably, enveloped viruses are the most sensitive to physicochemical parameters, including pH, temperature, salt concentration, and shear stress[22,50].

4. Quality control and regulatory aspects considerations

In contrast to most anticancer therapeutics, OV are unique because they are live and replicating viruses, and some are modified to carry transgenes. Thus, within biological medicinal products, OV-based therapies fall under either advanced therapy medicinal products (ATMP) or gene therapies, depending on the nature of the drug product[10,51].

Beyond their pharmaceutical classification, OV are intended to be administered intratumorally or intravenously, and therefore require a comprehensive panel of analytical assays. Firstly, sequencing, PCR-based assays, or immunoblotting are needed to validate product identity as well as potential genetic derivatives. Secondly, sterility and endotoxin testing, detection of adventitious agents and host cell–derived impurities, as well as tumor cell selectivity, are required to ensure product safety and purity. Finally, as infectivity is a central quality attribute directly linked to clinical efficacy, quantification of infectious virus is required by determining tissue culture infectious dose 50 (TCID50), plaque-forming units (PFU), or focus-forming units (FFU) [10,43]. Finally, in vivo testing is mandatory to validate the therapeutic efficacy, pharmacokinetics, biodistribution, and safety (including both tumorigenicity and tumor selectivity) of the OV-based therapy before clinical application[10,52].

The complexity of OV-based therapies in the anticancer therapeutics field, along with the diversity of viral platforms, has made the development of standardized regulatory guidelines challenging. This has led the FDA to issue a guidance document providing recommendations on regulatory standards for OV-based therapies in preclinical and clinical settings[53].

To support the development of OV-based therapies, a quality by design approach should therefore be implemented at early stages to establish scalable, cGMP bioprocesses, combined with rigorous and precise quality control to meet regulatory requirements.

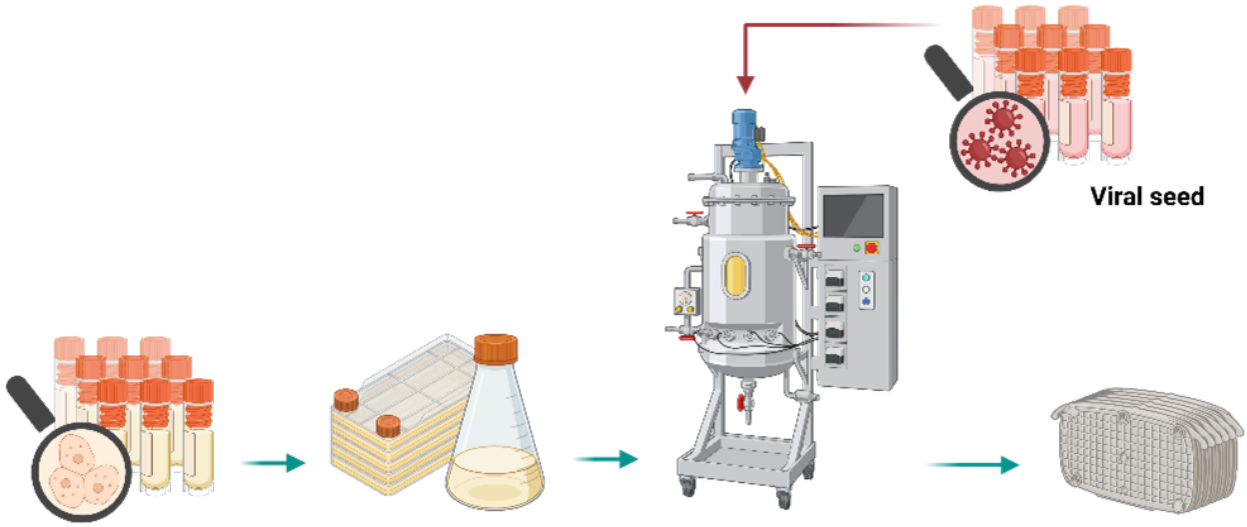
5. Oncolytic viruses market

The oncolytic virus market is still in an early stage but shows strong growth potential. It is expected to grow from about USD 3.7 billion in 2025 to over USD 11 billion by 2034, reflecting a compound annual growth rate (CAGR) of about 13%. Currently, only a few therapies are approved, including Imlygic (HSV-1), Oncorine (adenovirus), and Delytact (HSV-1). Despite the limited number of marketed products, the field is highly active, with many candidates in clinical development, suggesting significant expansion of the OV market in the coming years.

6. End-to-end manufacturing solutions for oncolytic viruses

Naobios supports the development and manufacturing of oncolytic viruses to accelerate the transition from research to clinical trials. Starting from the research virus seed and cell line, the team develops and optimizes robust upstream and downstream processes, while manufacturing fully characterized GMP master cell banks and virus banks. After process scale-up and industrialization, Naobios performs GMP manufacturing in its state-of-the-art facilities. With integrated capabilities from starting materials to drug product filling and quality control, Naobios provides an end-to-end solution to bring oncolytic virus projects rapidly to the clinic.

Upstream Process



Cell bank

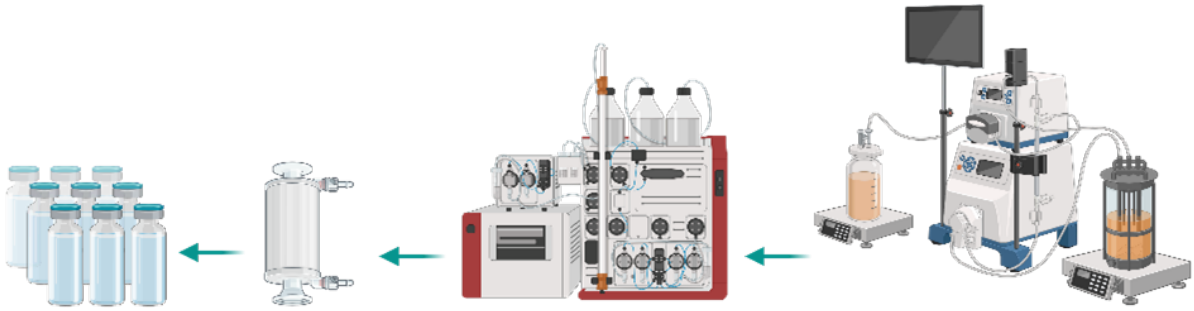
Cell amplification

Viral infection and production in (stirred-tank) bioreactor

Viral seed

Clarification
depth filtration

Downstream Process



Diafiltration and concentration
tangential flow filtration

Purification
liquid chromatography
(size exclusion, ion exchange)

Sterilization
0.2µm filtration

Fill & Finish

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