ATJ
Advanced Therapies Journal

VOL.6 / ISSUE 21 / AUTUMN 2024

Online ISSN: 2676-7236

DOI: 10.22034/atj.2024.210456

OPEN ACCESS

Pages: 9-19 / Review Article / Submit: 12 October 2024 / Accepted: 08 November 2024

Availablein: https://www.atjournal.ir

Introduction of Oncolytic Viruses as Candidates for Targeted Cancer Therapy

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Abstract

Given the progress in comprehending various forms of cancer and the subsequent pursuit of a remedy, along with improved survival rates for cancer patients, it is crucial to discover a therapeutic that may effectively counteract the aggressive mechanisms of this illness. Oncolytic viruses (OVs) have shown to be very advantageous in the treatment of cancer due to their ability to induce antitumor effects via several mechanisms. Viruses may be used to infect cancer cells, particularly in comparison to normal cells, to introduce tumor-associated antigens, trigger "danger signals" that create a less immune-tolerant tumor microenvironment, and function as delivery vehicles for the release of inflammatory and immunomodulatory cytokines. These modified OVs, which have been designed to have improved capacity to target tumors, increased oncolytic activity, or the potential to generate strong anti-tumor immune responses, are evaluated in animal models during preclinical testing and in clinical trials involving cancer patients. OVs have been recognized as one of the primary agents for cancer immunotherapy due to their ability to target tumors via many mechanisms. Nevertheless, given the restricted efficacy of innovative anti-cancer treatments including immunotherapies and cell-based therapies, it is imperative to evaluate the potential of combination therapy using OVs. This study aims to introduce oncolytic viruses and review their capacity to induce antitumor responses, their challenges and limitations.

Keywords: Cancer treatment, Oncolytic viruses, Tumor lysis, Therapy.

Introduction

For the past two decades, oncolytic virotherapy, a new cancer treatment method, has yielded encouraging outcomes. More than a century ago, it was discovered that cancer patients had cancer regression if they were infected with certain viruses. Revolutions in recombinant DNA technology have offered key tools for studying viral biology, enhancing biological treatment for cancer, and ushering in a new generation of cancer therapies. While chemotherapy and radiation therapies remain popular cancer treatments, their severe side effects are a big disadvantage (1). Biological therapy for cancer, despite its complexity and difficulty, is the preferred treatment choice because of its success, low negative reactions, and decreased discomfort for cancer patients. So far, research studies have found no fatalities or clinically significant side

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How to Cite this Article:
A.S. Ahmadi, Z. Zand. " Introduction of Oncolytic Viruses as Candidates for Targeted Cancer Therapy", Advanced Therapies Journal, vol. 6, no. 21, pp. 9-19, 2024.

effects related to oncolytic virotherapy (OV). In cancer treatment, patient safety is critical, and treatment using oncolytic viruses appears to be the most promising in this regard. Most oncolytic viruses used in cancer therapy are attenuated strains, which may infect and multiply in people without causing major sickness. It is also critical that the viruses picked can employ the host immune system to identify and attack cancerous cells (2).

Oncolytic viruses (OVs) have gained popularity in recent years due to their capacity to specifically target and kill tumour cells while potentially stimulating antitumor immunity. There are several benefits to developing tumours that specifically target OVs while maintaining a tolerable safety profile and effectiveness against various malignancies. A candidate virus chosen to eliminate tumour cells must feature specific hallmarks: it must be proimmunogenic, not cause chronic infectious illness, be safe for diverse human populations, exert lytic activity in tumour cells, and have the power to integrate into the host genome (3). An oncolytic virus's proimmunogenicity is defined as the presence of antigenicity, which allows the immune system to recognise it, infectivity, replication, which causes a powerful immunological response, and genetic diversity, which can contribute to immune recognition and inflammation. Genetic engineering is critical in creating OVs with a tolerable safety profile and excellent effectiveness against a variety of malignancies. In this sense, OVs must be adjusted especially for the tumour type and mutations by changing noncoding mutations and adding or deleting certain activities, genes, and noncoding elements to impart extra positive qualities. There are three research directions for cancer therapy with OVs: Viral replication, tumour development, and immune activation. As a result, OVs primarily target tumour cells, host immunity, and the tumour microenvironment. Most viral vectors cause immunogenic cell death (ICD) by releasing tumor-associated antigens, which trigger antitumor immune responses. By stimulating antitumor immunity, OVs outperform ICIs and other precisely targeted medicines. OVs have a wide variety of anticancer, immune-boosting effects. Patients with acceptable molecular profiles must be identified before receiving OV treatment. Thus, the identification of prognostic biomarkers for OV therapy is critical. We will look at many features of OVs and their usage in oncolytic virotherapy. We think that oncolytic virotherapy provides a novel approach to cancer immunotherapy (4, 5).

Types of viruses used for oncolytic virotherapy

An optimal oncolytic virus candidate should have certain characteristics, including a strong fundamental grasp of their biology and genetics. The OV should be pro-immunogenic, have lytic action in infected malignant cells, not cause chronic or infectious illness, and be able to integrate into the human genome. Furthermore, the virus must be generally safe for a varied human population. It is also possible to genetically edit and arm with recombinant transgenes to increase its immunogenicity or induce specific anticancer processes. From the initial investigations, a variety of viruses with and without genetic changes have been investigated and joined clinical studies (6). They are composed of RNA as well as DNA viruses (Table 1). Oncolytic DNA viruses include adenoviruses, herpes simplex virus (HSV), parvoviruses, and poxviruses including vaccinia (VACV) and myxoma (MYXV). Oncolytic RNA viruses include Coxsackie virus, Maraba virus, measles virus (MV), Newcastle disease virus (NDV), poliovirus, reovirus, retroviruses, Seneca Valley virus (SVV), Semliki Forest virus (SFV), Vesicular Stomatitis virus (VSV), and Sindbis virus (SBV). Oncolytic DNA viruses offer the benefits of high genome stability and greater transgene insertion capabilities without impacting viral infection and proliferation. On the other hand, RNA viruses have limited genome-packing ability, yet some can be highly immunogenic. However, there are advantages and disadvantages to each OV virus that has been produced and tested thus far (7).

Once OVs attach to and invade tumor cells, they may use many destructive processes to eliminate the infected cancer cells. The effectiveness of these mechanisms may or may not be directly related to the level of viral replication occurring inside the target cells. The precise processes behind viral oncolysis remain partially known, exhibiting significant variation between different viruses and even substantial differences across various kinds of cancer cells being targeted (8). OVs are believed to exert antitumor effects through various mechanisms: (a) they replicate selectively within cancer cells, resulting in direct destruction of the cells (also known as oncolysis); (b) they induce cell death, either through apoptosis-like or necrosis-like processes, in both infected and uninfected cancer cells as well as the endothelial cells in the tumor-associated blood vessels, leading to decreased formation of new blood vessels (angiogenesis); and (c) they stimulate a systemic immune response against both the tumor and the virus, attracting activated immune cells into the tumor microenvironment (TME). Nevertheless, these pathways exhibit substantial variations between different viruses, as well as about the specific characteristics and types of cancer cells, and the overall interplay between the OVs, TME, and the host immune system (9). Many viruses inhibit the cell death pathways that are triggered by the host in response to viral infection.

Table 1. A list of oncolytic viruses and their characteristics.

	Name	Size of genome	Capsid symmetry	Envelope	Entry receptor	Host	Site of replication
DNA virus	Adenovirus	dsDNA 70–100	Icosahedral	Naked	CD46, CAR	Human, animals	Cytoplasm and nucleus
	Herpes Simplex Virus-1	dsDNA 200	Icosahedral	Enveloped	Nectin 1,2, HVEM	Human (HSV-1)	Cytoplasm and nucleus
	Vaccinia Virus	dsDNA 70–100	Complex	Complex coat	Without specific receptor	Humans and cattle	Cytoplasm
	Poxvirus: VACV, MYXV	dsDNA (160–190 kb)	-	Enveloped	Heparan, laminin, chondroitin, integrin β1	MYXV (rabbit)	Cytoplasm
RNA virus	Measles	ss(-)RNA 100–200	Icosahedral	Enveloped	CD46, SLAM	Human	Cytoplasm
	Vesicular Stomatitis Virus	ss(-)RNA 80	Helical	Enveloped	LDRL	Humans, mammals, and insects	Cytoplasm
	Newcastle virus	ss(-)RNA 100–500	Helical	Enveloped	Sialic acid	Birds	Cytoplasm
	Reovirus	dsRNA 60–80	Icosahedral	Naked	Without specific receptor	Human	Cytoplasm
	Zika virus	SS (+) RNA (10.8 kb)	Icosahedral	Enveloped	GAGS, Heparan sulfate, C-type lectin	Monkey	Cytoplasm

1.Antitumor effects induced by OVs

Viruses have been seen to produce proteins that may selectively affect several routes of cell death, acting as either inhibitors or inducers. Once infected by an OVs, the cancer cells often undergo cell death due to the activation of cell death pathways and/or the breakdown of cell integrity caused by the damage inflicted by the virus. In addition, several OVs have been modified to selectively trigger certain cancer cell death mechanisms, such as apoptosis, necrosis, autophagy, or pyroptosis, in order to enhance cell lysis (10). ICD refers to a specific type of cancer cell death that allows cancer cell antigens to be exposed to immune cells in the TME. In laboratory settings, ICD is typically measured by observing the external exposure of markers that are normally found inside cells or the release of internal mediators by the dying cells. OVs have the benefit of being able to activate many cell death pathways inside the tumor site. Out of them, it is considered that ICD plays a vital role in enhancing acquired anti-tumor immunity (11).

When the replication of OVs in cancer cells triggers ICD, it leads to the release of tumor-associated antigens (TAAs), damage-associated molecular patterns (DAMPs), OV-derived pathogen-associated molecular patterns (PAMPs), and increased production of various inflammatory cytokines. These factors collectively stimulate both the innate and adaptive immune responses. The presence of DAMPs, including extracellular ATP and high mobility group box 1 (HMGB1) proteins, as well as the exposure of certain cytoplasmic proteins on the cell surface, such as HSP70, HSP90, and calreticulin (CRT), are all characteristic signs of ICD. Following the release, DAMP molecules attach to their corresponding receptors CD91 (CRT), P2RX7 (ATP), and TLR4 (HMGB1) on dendritic cells (DCs). This interaction leads to the maturation of DCs, antigen processing, and the subsequent activation of T lymphocytes, resulting in improved antitumor responses. Extracellular adenosine triphosphate (ATP) and surface-exposed calreticulin (CRT) function as signals that attract and facilitate the engulfment of phagocytic immune cells. Cytoplasmic DNA in infected cells triggers the cGAS DNA sensor at the molecular level, leading to the activation of STING pathways. This activation results in the initiation of innate immunity through

the expression of type I IFN genes, the release of chemokines CXCL9 and CXCL10, and ultimately the recruitment of T cells (12). ICD, or immunogenic cell death, plays a crucial role in the establishment of anticancer immunity in metastatic locations in the context of oncolytic virotherapy. Recent research has shown that OVs, such as adenovirus, parvovirus, reovirus, coxsackievirus, vaccinia virus (VACV), Newcastle disease virus (NDV), and herpes simplex virus (HSV), all elicit different levels of immunogenic cell death (ICD). The activation of ICD by oncolytic viruses (OVs) is essential for transforming cancers that lack lymphoid cells or have minimal expression of immunological sensors (referred to as "cold" tumors) into tumors that are infiltrated by T cells and have an inflammatory immune microenvironment (referred to as "hot" tumors). In addition to ICD, autophagy may also trigger antitumor immune responses as a result of OV infection and replication inside cancer cells. For instance, the stimulation of autophagy increased the reproduction of oncolytic Adenoviruses and NDV. Autophagy also increased the effectiveness of tumor destruction by oncolysis, autophagic cell death, and immunogenic cell death (12).

OVs are capable of targeting specific genes

OVs employ gene targeting as a significant antitumor mechanism. For example, reoviruses capitalize on specific cellular characteristics that are frequently altered in cancer cells. The signaling of specific proteins, including TP53, PTEN, RB1, and RAS, is modulated in tumor cells, resulting in the activation of tumorigenesis, angiogenesis, invasion, and metastasis, as well as the inhibition of apoptosis (12). This is achieved by the activation of oncogenes and the inactivation of tumor suppressor genes. OVs modulate a variety of signaling pathways in tumor cells by targeting these signaling cascades. The action of reoviruses on tumor cell cultures has been investigated, and viral selective replication steps within the tumor cells have been identified. After reovirus infection, the Ras mutation can facilitate reovirus entry and oncogenic transformation, increase the production of OVs, induce apoptosis, and prevent interferon production, thereby facilitating the virus's dissemination as a result of a defective antiviral response. Reovirus enters cells by binding to particular receptors on the cell surface (13). The virus's capacity to enter and infect cancer cells may be enhanced by alterations in the expression or accessibility of these receptors. Oncolytic VVs and VSVs selectively infect tumor cells and induce endothelium death by exploiting the overexpression of ras/mitogen-activated protein kinase (MAPK). Some viruses, like reovirus, have an inherent inclination towards tumor cells, whereas other viruses, such as Ads, VSVs, and HSVs, need to be modified to specifically target tumor cells (14). Healthy cells that are infected with OVs prevent the spread of the virus via several mechanisms that are often lacking in tumor cells. The antiviral capability of a cell, which is connected with type I interferon, is linked to the preference of a vesicular stomatitis virus (VSV) for cancer cells. Typically, normal cells generate type I interferons (IFNs) to hinder viral replication. However, most tumor cells have impaired type I IFN production, making them susceptible to VSV infection (15).

Changing and interrupting the vascular environment at the tumour

Tumor angiogenesis is influenced by the expression of proteins regulated by oncogenes and by factors that cause cellular circumstances, such as low pH, hypoxia, nutritional shortage, or the production of reactive oxygen species (ROS). The antiangiogenic methods of OVs are as outlined below: (i) The tumor cells are directly infected and the newly formed blood vessels are destroyed; (ii) The immune response is activated by the OVs, causing cells to clump together and slowing down blood flow; (iii) The viral proteins produced by the OVs prevent the production of factors that promote the growth of blood vessels in the tumor. Direct antivascular characteristics are a common characteristic of several Ovs (16). OBP-301, an adenovirus engineered with human telomerase reverse transcriptase components that regulate E1 gene expression, was developed to generate IFN-gamma, a cytokine with antiangiogenic properties. This cytokine selectively destroys cancer cells in murine colon cancers in syngeneic animals. Viral Stomatitis Virions (VSVs) induce coagulation and provoke an inflammatory response inside the circulatory system (17). The infection with VV leads to the disruption of the EGFR/Ras signaling pathways, causing vascular leakage and collapse. The OV iNDV3a-LP improves the destruction of endothelial cells. Systems using Armed VV-, HSV-, MV-, and Ad-based approaches regulate the amounts of endostatin and angiostatin, leading to the collapse of blood vessels. Furthermore, the interaction between VEGF and antiangiogenic proteins may lead to the downregulation of VEGF. This downregulation then causes damage to newly formed blood vessels in the TME, eventually resulting in the destruction of cancer cells (oncolysis) (18, 19).

Methods of transporting OVs

Direct deliver

Intratumoral administration

The intratumoral method is often used to administer OVs in clinical studies. Multiple experiments have shown the efficacy of delivering drugs directly into tumors and have investigated its potential to

maintain sufficient levels of OVs by bypassing the bloodstream. The local method of administering the medication directly into the affected area helps to reduce the deactivation of the virus. The limited engagement of the innate immune system with the virus minimizes the chances of systemic toxicity and transmission of the targeted viral load during a distinct injection method (20). The drawbacks of this approach are the inability to reach a therapeutic level in the tumor because of a thick extracellular matrix (ECM) and the insufficient delivery of the treatment inside the tumor due to the formation of new blood vessels (neoangiogenesis). Systemic reactions may be triggered by the intratumoral administration of OVs. Tumor antigens are produced as a result of the interaction between OVs and tumor cells. Research on the use of T-VEC has shown a reduction in the dimensions of the examined melanoma growths, including those that were directly treated with the injection and those that were not (21).

Intravenous administration

Intravenous administration offers advantages for treating malignancies at the metastatic stage, particularly when there is a need to target several tumors of varying sizes and locations. It is also beneficial in situations when intratumoral administration is not possible due to the tumor's position. This delivery method may effectively enhance the dispersion of OVs throughout the tumor mass. However, the precise dosage of OVs that yields the best results is yet unknown (22). Certain OVs can elicit a heightened immunological response when given intravenously. The immune system employs many techniques to impede the effective dissemination of OVs to their intended target tissues. OVs can bind indiscriminately to serum proteins or circulating cells in the bloodstream, leading to their ultimate death. Since these processes are components of the innate immune system, they are also efficient in individuals who have not been exposed to a specific virus (21, 22). Additionally, a previous exposure may lead to substantial elimination of the virus due to the existence of a specific and robust immune response. Antibodies may be removed by either complement action or destruction by macrophages in various organs when they attach to virus particles. The liver, spleen, and lungs are the primary organs responsible for the neutralization of OVs. The tumor ECM and interstitial fluid pressure provide additional challenges to the successful intravenous delivery of OVs. The high interstitial fluid pressure restricts entry into the core of the tumor, where it is at its maximum due to the fluid flow around its edges. However, it also hinders the movement of big molecules (23, 24).

Transporting Ovs systemically via cargo

There have been several diverse investigations conducted on the subject of OV cargo delivery to tumors, which may be categorized into biological and nonbiological delivery methods. Biological carriers for oncolytic viruses (OVs) comprise cellular carriers, carriers mediated by cell membranes, and carriers mediated by serum albumin. Nonbiological vehicles for OVs contain mineralized nanostructures, magnetic nanostructures, and polymer-based nanocarriers (25).

Biological carriers of oncolytic viruses

Cellular Vectors

Mesenchymal stem cells (MSCs) have garnered growing interest in scientific research due to their unique biological characteristics, broad therapeutic potential, and influence on tissue engineering. MSCs demonstrate this ability by transforming into osteogenic, adipogenic, or chondrogenic tissues and possess an extensive network for secreting various mediators, cytokines, and signaling molecules. Furthermore, they possess notable characteristics of self-renewal and multipotency. This secretion modulates the inflammatory response and regulates essential infiltration processes necessary for tissue regeneration and repair (26). The regulation of MSCs is influenced by feedback mechanisms occurring inside the axis formed by the MSC molecule and the target cell. MSCs cannot exhibit costimulatory molecules, resulting in reduced immunological activity. This makes them suitable for use in cell-based immunotherapy for malignancies. MSCs may enhance the in vitro multiplication of oAds, promoting the transport and long-term survival of viruses by inhibiting immune responses specific to the virus in a rat model. In addition, as compared to viral injection alone in living organisms, CR-MSCs have the potential to reduce the production of IFN by activated T cells, resulting in a larger enhancement of both the dispersion and survival of oAds (27).

When mesenchymal stem cells (MSCs) infected with oAds were given to mice with hepatocellular carcinoma, the virus particles built up in the tumor and effectively stopped its growth. A further research study used human MSCs to carry a replicative Ad. This Ad had an E1A gene that was driven by the alpha-fetoprotein promoter. The study demonstrated significant inhibition of growth in both orthotopic and subcutaneous hepatic xenograft cancer mouse models. After being administered systemically, MSCs that were infected with an oncolytic adenovirus (oAd) showed therapeutic effects in mice with lung and breast cancers that were implanted in their natural locations. These effects resulted in an improvement in survival rates (28). Systemic administration of human MSCs modified with replicating viruses,

namely Ad5/3.CXCR4 and Ad5R6D. CXCR4 Ads, in experimental models of metastatic breast cancer, resulted in improved animal survival. Administration of MSCs that were infected with a HER2-retargeted oncolytic HSV resulted in the dissemination of the virus to breast and ovarian cancer cells in laboratory models. This was followed by a decrease in the spread of cancer cells to the lungs and brain. Investigation on SKOV3ip. An ovarian tumor xenograft study shown that intraperitoneal administration of MSCs laden with oncolytic MeVs in SKOV3ip resulted in positive outcomes. A single ovarian tumor xenograft can infiltrate tumor lesions and subsequently transmit viral infection in mice, resulting in an overall increase in the average lifetime. The use of CRAd5/F11 chimeric oAds specifically targeting MSCs resulted in a considerable suppression of colorectal cancer cell proliferation (29).

Extracellular vesicles (EVs)

Extracellular vesicles are membranous vesicles that are secreted by different cell types and play a role in intracellular communication by transporting lipids, proteins, RNA, DNA, and carbohydrates. These vesicles are categorized based on their size as exosomes, microvesicles, apoptotic bodies, and oncosomes (30). They are often used for transporting antineoplastic medications, such as oncolytic viruses (OVs). Garofalo et al. found that the combination of Ad5D24-CpG and PTX enclosed in extracellular vesicles (EVs) caused inflammation around the tumor, leading to improved effectiveness and toxicity against lung cancer cells both in laboratory settings and in living organisms. Exosomes originating from tumor cells often exhibit immunosuppressive properties. However, exosomes obtained from tumor cells infected with oncolytic viruses (OVs) may possess immunostimulatory properties. Labani-Motlagh et al. demonstrated that melanoma cells, when infected with oncolytic viruses (OVs) that were equipped with CD40 (CD40L) and 4-1BB (4-1BBL) ligands, resulted in increased activation of dendritic cells (DCs) and improved responses in the tumor microenvironment (TME) (31). Ovarian cancers may include exosomes, which can preferentially aggregate in tumors and exert anticancer effects either locally or in distant malignancies. The adenoviruses were specifically engineered to carry immunostimulatory transgenes and were intended to be loaded for therapeutic purposes. The tumor cells infected with LOAd carry the transgenes, which are enclosed inside the endosomal compartments and then released as exosomes. Furthermore, the levels of mRNA were notably elevated in exosomes obtained from cells infected with both LOAd700 and LOAd703 (32). It was noted that every infected cell can produce the transgenes, regardless of whether the virus exclusively replicates in tumor cells. The immature dendritic cells (DCs) were then infected with the LOAd viruses and then stimulated in a laboratory setting using exosomes. OVs arm the exosomes formed from arm tumors with immunostimulatory transgenes, which may contribute to the mechanism behind the ability of local therapy to induce systemic immunity. Furthermore, it was shown that the transportation of microRNA (miRNA) into exosomes is modified in cells that are infected (33).

Delivery of OVs throughout the body via inorganic vehicles

Several different kinds of nanoparticles (NPs), including liposomes, dendrimers, gold nanoparticles, silica nanoparticles, and iron oxide nanoparticles, may be connected to OVs. There are two approaches for the transportation of OVs employing NP carriers. The first approach involves the use of shielding and surface modification techniques, while the second approach focuses on the combined treatment of OV-NPs. There are two methods for transporting NPs to the tumor location, namely active and passive processes (34). The passive process involves the enhancement of tumor blood vessel permeability via the EPR effect, resulting in the concentration of NPs in the tumor area. Active delivery refers to the specific attachment of NPs to target cells, to raise the concentration of the therapeutic drug in cancerous tumors while minimizing adverse effects. Peptides, proteins, aptamers, and polysaccharides are examples of biological ligands that can bind specifically and with high affinity to molecules or receptors found in tumor cells (35).

Advancements in combination therapy and the drawbacks of single therapy

While several tumor-selective pathways have been shown for OVs in both preclinical and clinical settings, their effectiveness was restricted when used as standalone treatments. There are several possible explanations for the limited effectiveness of systemic OV monotherapy. Initially, the presence of antiviral antibodies, whether from therapy or preexisting, may impede the replication and destruction of tumors by OVs. Additionally, antiviral resistance mechanisms, including complement activation, antiviral cytokines, and macrophages, may facilitate the rapid elimination of Ovs (36). The presence of antiviral immunities may provide a significant obstacle for OVs. However, the impact of antiviral immunity is not well understood, and the use of intratumoral OV treatment may potentially overcome this challenge by exerting local and abscopal effects. Remarkably, preexisting immunity to NDV restricts its ability to reproduce in tumors. However, this does not hinder tumor elimination, abscopal antitumor

immune responses, or survival. These outcomes are enhanced in mice that have been inoculated with NDV and received repeated therapeutic doses (37). These findings provide a clinical justification for the use of repeated dose treatment. Furthermore, the presence of the extracellular matrix, fibrosis, necrosis, and interstitial hydrostatic pressure may create a formidable physical obstacle that prevents OVs from accessing cellular receptors located in tight junctions. This difficulty has garnered significant interest among researchers who are seeking ways to overcome it (38). Furthermore, the expression or manipulation of transgenes to achieve tumor selectivity might result in a decline in viral fitness, leading to a decrease in replication capability and oncolytic effectiveness. Furthermore, the activation of transgenes might trigger the elimination of OVs due to a significant immunological response, hence facilitating ongoing refinement and modification of transgenes. Due to the limited effectiveness of using a single drug, research has mostly concentrated on choosing both a virus and a combo partner (38).

Therefore, the use of combination treatment with OVs has become a compelling choice. Moreover, the processes of OVs are fundamentally unlike those of other anticancer therapies, and the toxicity profiles often do not coincide with those of other treatments (39). Additionally, OVs may be injected many times if necessary. OVs possess characteristics that make them a logical choice for stimulating individualized immune responses and integrating them with various other treatment approaches, such as chemotherapies, radiotherapy, targeted therapies, and immunotherapies like immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR) T-cell therapies, and adoptive T-cell therapies. When evaluating the advantages of combining the OV with another agent, it is important to consider various factors. These factors include comprehending the inherent lytic and immune-modulatory properties of the virus, as well as considering the site of action, duration of therapy required, and cost of goods. Multiple studies have examined the combined effects of OVs with chemotherapy or radiation to assess their synergistic impact (40).

A recent review provided a summary of the important molecules involved in signaling pathways that are relevant to the study, such as EGFR-KRAS (specifically KRASG12C), PI3K-AKT-mTOR, ERK-MEK, JAK-STAT, p53, PD-1-PD-L1, and epigenetic or immune pathways (including histone deacetylases, cGAS-STING). These pathways are currently being investigated and have the potential to be combined with OV (41). Conversely, the activation of a widespread immune response by OVs to convert non-responsive cancers into responsive tumors might enhance the effectiveness of immunotherapy methods like immune checkpoint inhibitors (ICIs). Genetically modifying OVs and combining them with CAR-T-cell treatment may effectively enhance the ability of CAR-T cells to identify and infiltrate tumors. These combinations have the potential to effectively address the weaknesses of each component, hence improving the overall output (42).

The most sophisticated combination treatment protocols now being used in medical practice use ICIs, and early evidence indicates promising results. The progress in the discovery of ICIs has revolutionized the landscape of contemporary cancer therapy. OV infection may result in the increased expression of immune cells and immunological checkpoint markers inside the TME (43). Liu et al. equipped OVV with IL-2 to efficiently alter the cancer-immune equilibrium, and when combined with an anti-PD-1/PD-L1 antibody, it successfully eradicated the majority of advanced tumors in mice (43). Bo et al. discovered that oncolytic HSV2 increases the expression of PD-L1 in the TME (44). Furthermore, it has been shown that OVs may increase the expression of immunological checkpoint molecules largely found on natural killer (NK) cells, in addition to those produced on T cells and tumor cells. According to Wang et al., they found that oncolytic HSV2 increases the expression of NKG2A in NK cells. They also discovered that using anti-NKG2A antibodies enhances the anticancer effects of UV light-inactivated oHSV2-stimulated NK92 cells, both in laboratory tests (in vitro) and in living organisms (in vivo) (45). Nakao et al. discovered that the intratumoral production of IL-7 and IL-12, facilitated by an oncolytic virus, enhances the body's response to immune checkpoint suppression, hence increasing systemic sensitivity. Increasing the expression of immune checkpoint molecules may provide potential targets for combining with immune checkpoint drugs in clinical trials (45). Extensive phase III studies have shown that oncolytic viruses have a significant role in both destroying cancer cells and inducing beneficial changes in the tumor immune microenvironment. "Priming" by viral infection may transform the "cold" TME into a "hot" one, characterized by the presence of many immune cells and cytokines. This sets the stage for the effective delivery of future ICI treatment. The quantity of clinical studies investigating the combination of OVs with ICIs is steadily increasing. Initial findings from these trials indicate encouraging therapeutic possibilities with favorable safety profiles (46).

T-VEC is at the forefront of this very promising combo immunotherapy. The phase Ib stage of the MASTERKEY-265 trial demonstrated encouraging tumor responses when T-VEC was combined with pembrolizumab. Additionally, a phase 1b clinical trial demonstrated a confirmed objective response rate of

62%, with a complete response rate of 33% when using the combination of T-VEC and pembrolizumab. This indicates that T-VEC has an effect on the infiltration of cytotoxic T-cells and improves the effectiveness of ICI therapy by modifying the TME (47). Sun et al. conducted a review of a case series and found that the combination of T-VEC with ICIs (including pembrolizumab, ipilimumab/nivolumab, or nivolumab) resulted in an overall response rate of 90% for the treatment of unresectable stage III-IV melanoma (48). This indicates that the combination therapy may have synergistic effects and be beneficial for patients. ONCOS-102, a kind of Ad5 virus that has been equipped with GM-CSF, has shown encouraging results in fighting tumors when used along with pembrolizumab in patients with advanced or inoperable melanoma (48).

Challenges associated with oncolytic virotherapy

Similar to other contemporary cancer treatments, oncolytic virotherapy also faces several problems and barriers on its path to becoming an effective anticancer therapy. The limitation of OV functions can be attributed to several key factors. Firstly, the presence of unknown host antiviral pathways restricts the activity and spread of OV in the tumor bed. Secondly, intrinsic physical barriers in the tumor bed hinder the access of OV. Lastly, adaptive immune responses indirectly limit the viral function (4). Moreover, there are supplementary considerations that must be taken into account:

1.Best choice of an OV candidate: So far, many DNA and RNA viruses have been investigated as possibilities for OVs. To be considered an ideal candidate, the selected virus must possess several key characteristics (45). These include the capacity to stably incorporate transgenes, minimal or no toxicity towards normal cells and tissues, low immunogenicity, the ability to be amplified on a large scale for clinical use, optimization of production, and appropriate therapeutic targets for the chosen Ovs (49).

2.Process of viral entrance, infection, and dissemination: OVs that rely on certain cell surface receptors for attachment and entry are often ineffective against cancers that have diminished or absent expression of such receptors. While engineering has successfully addressed this obstacle for some viruses, a small number of viruses (such as poxviruses) may bypass this problem by attaching to non-specific factors like widely distributed cell surface glycosaminoglycans (50). At the cellular level, there are intricate signaling channels that are connected, either directly or indirectly, to the antiviral pathways. These pathways often hinder the reproduction and transmission of viruses to new cells. AKT activation levels directly control the replication of MYXV in human cancer cells. Excessive ECM in the tumor bed hinders the propagation of viruses. For instance, the presence of fibrillar collagen in the ECM restricts the spread of oncolytic HSV inside tumors (50).

3.Delivery: Delivery of OV to both the main and metastatic locations is crucial for achieving the best treatment results. Regarding this matter, since only a small portion of human malignancies may be effectively treated by directly injecting the virus into the tumor, systemic delivery is the preferable method over intratumoral (IT) injection (45). Nevertheless, several obstacles impede the effective implementation of any OV. Neutralizing antiviral antibodies, complement activation, generation of antiviral cytokines, and the liver and spleen's natural clearance site for OVs all provide significant challenges for the systemic distribution of OVs. While IT delivery of viruses may overcome some obstacles, the dissemination of viruses can be restricted by tumor beds, and the tumor vasculature also poses limitations for IT and metastatic locations. An effective approach to address these problems is to use migrating leukocytes as carrier cells to transport the virus into tumor beds that permit cellular entry (51).

4.Antibodies that neutralize viruses and cytokines that combat viral infections: The primary challenge in delivering free virus systemically to the tumor site is the presence of preexisting neutralizing antiviral antibodies (48). In addition, the immune system of the host triggers antiviral immunity and restricts the oncolytic action of oncolytic viruses. The cellular receptor molecules responsible for detecting virus particles and virus-infected cells initiate type I IFN signaling pathways, which in turn activate antiviral defense pathways in uninfected cells. This process helps to restrict the infection and spread of the virus. Furthermore, the immune system's ability to eliminate infected cells, including cancer cells, not only stops the transmission of the virus but also plays a crucial role in triggering anti-tumor immune responses (52).

5.Immunosuppressive tumor microenvironment: Another obstacle to OV treatment is the common occurrence of a highly immunosuppressive TME. Within the area where the tumor is located, many types of cells such as cancer cells, stromal cells, inhibitory cytokines (such as TGF-beta), and immune cells that invade the tumor (such as regulatory T cells and myeloid-derived suppressor cells) all have a role in creating an immunosuppressive tumor microenvironment. While it is crucial for the tumor to avoid the host's natural and acquired immune system defenses, OVs must nonetheless operate inside this immunosuppressive TME (52). In addition, some OV infections may enhance the immunosuppressive environment of the tumor bed by stimulating the immune system. As an example, the Maraba virus increased the activity of the PD-1/

PD-L1 pathway on both tumor cells and immune cells that had infiltrated the tumor. Likewise, the oncolytic NDV virus induced the development of PD-L1 at the tumor site via the activation of type I IFN signaling. This led to the creation of an immunosuppressive tumor microenvironment, even in tumors located far away (52).

Conclusions

Oncolytic viruses have emerged as a strategy to overcome the tumor's ability to evade the immune system. The goal is to enhance the clinical state of patients by either stimulating the host immune system or directly destroying aberrant cells. Advancements in genetic engineering have enabled the enhancement of OVs, resulting in improved safety and effectiveness. These advancements allow for the precise targeting of the virus to the tumor and a reduction in the negative side effects associated with its usage. Moreover, it is feasible to detect substantial impacts of the therapeutic use of OVs, whether used alone or in combination therapy, on the treatment of malignancies. Hence, enhancing anticancer treatments and subsequently enhancing patient prognosis via the integration of molecular biology, structural biology, immunology, genomics, and bioinformatics establishes a strong basis for the future clinical effectiveness of OVs.

The authors would like to thank the Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, 1417613151, Iran for their support.

Authors's Contribution

Akram Sadat Ahmadi and Zahra Zand were involved in the conceptualization, design and writing of the manuscript draft. All authors read and confirmed the final manuscript.

Funding

The author of this article has not received any financial support from private or government sources.

Ethics approval and consent to participate

Not applicable

Conflict of Interest

The authors declared no conflict of interest.

Consent for publication

Not Applicable

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