Current landscape and perspective of oncolytic viruses and their combination therapies

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ABSTRACT

Oncolytic virotherapy has become an important strategy in cancer immunotherapy. Oncolytic virus (OV) can reshape the tumor microenvironment (TME) through its replication-mediated oncolysis and transgene-produced anticancer effect, inducing an antitumor immune response and creating favorable conditions for the combination of other therapeutic measures. Extensive preclinical and clinical data have suggested that OV-based combination therapy has definite efficacy and promising prospects. Recently, several clinical trials of oncolytic virotherapy combined with immunotherapy have made breakthroughs. This review comprehensively elaborates the OV types and their targeting mechanisms, the selection of anticancer genes armed in OVs, and the therapeutic modes of action and strategies of OVs to provide a theoretical basis for the better design and construction of OVs and the optimization of OV-based therapeutic strategies.

Introduction

Oncolytic viruses (OVs) are a kind of natural or artificially engineered viruses that can specifically replicate in cancer cells and eventually lead to cell lysis. OVs can not only be used as oncolytic agents alone, but they can also be used as effective carriers of anticancer genes and play multiple functions simultaneously such as virotherapy and gene therapy. Currently, OVs have attracted wide attention and are also considered as an important branch of cancer immunotherapy [1]. The discovery of OVs came from an accidental clinical observation that tumor spontaneously regressed after infection with influenza virus in a leukemia patient. Then, through a century of clinical research and genetic engineering, the artificially engineered viruses obtained more accurate tumor targeting and superior anticancer activity, with their anticancer mechanisms have been gradually elucidated. In 1996, a

genetically modified oncolytic adenovirus (OAV), ONYX-015, was first introduced into clinical trial [2]. In 2004, a nonpathogenic enteric cytopathic human orphan virus (Rigvir) was approved in Latvia for the treatment of melanoma, which was the first OV to be approved for cancer treatment worldwide [3]. A genetically modified recombinant human adenovirus type 5, Oncorine or H101, was approved for marketing in China in 2005 [4]. In 2015, the Food and Drug Administration (FDA) of USA approved T-vec (Talimogene laherparepvec, Imlygic) for the topical treatment of unresectable skin, subcutaneous and lymph node lesions in patients with recurrent melanoma after the first surgery. This was the first OV drug approved by the FDA. Subsequently, T-vec was approved in Europe and Canada, marking a milestone in oncolytic virotherapy for cancer [5]. On June 11, 2021, Daiichi Sankyo Company Limited of Japan announced that their oncolytic herpes simplex virus (HSV) Delytact (TeserpatureV /G47Δ) has received conditional,

time-limited approval from Japan's Ministry of Health, Labor and Welfare (MHLW) for the treatment of malignant glioma. This was the fourth OV approved worldwide and the first to be approved for malignant glioma [6]. There are currently four OVs that have been approved globally.

Compared to traditional chemotherapy and radiotherapy, OVs lyse cancer cells, without damaging normal cells, tissues, and organs. Therefore, oncolytic virotherapy shows significant potential to achieve efficacy for various types of cancers and improves the overall survival in cancer patients, which has been considered to be an immeasurable anticancer therapy, giving hope to cancer patients [7]. In this review, the research and development status and application of OVs are discussed in detail.

Viral types of OVs

Cancers are naturally good targets for OV attacks. When mutations occur in genes such as P53, RB1, PTEN, DCC, RAS, P16, and VHL, the antiviral infection capacity of cancer cells is weakened at the same time [8]. Many kinds of viruses in nature have potential therapeutic effects on cancers and can be modified into OV drugs. The top five commonly used OVs are adenovirus, herpes virus, reovirus, cowpox virus, and Newcastle disease virus. Adenovirus type 5 (Ad5) has been widely used due to its simple genome and in-depth studies [9]. According to whether the virus has been artificially modified, OVs can be divided into the natural weak virus strains (or wild type oncolytic viruses) and the genetically modified virus strains. Some natural virus strains such as reovirus, Newcastle disease virus, and vesicular stomatitis virus have a preference for tumor cells. However, natural OVs have limited efficacy in killing tumor cells, and are easy to activate the host immune system; hence, it is difficult to effectively control viral pathogenicity. The genetically engineered OVs were weakened in their killing ability to host normal cells through gene mutation or gene element regulation. They are endowed with targeted selectivity to cancer cells and enhance their anticancer activity by inserting exogenous genes. Therefore, the safety and effectiveness of this type of genetically engineered OVs are improved. Virtually all kinds of viruses, including HSV, adenovirus, vaccinia virus, measles virus, parvovirus, poliovirus, Malaba virus, reovirus, coxsackie virus, vesicular stomatitis virus, Newcastle disease virus, and picornavirus, can be engineered to recombinant OVs [10].

Each virus has its unique strengths and weaknesses. Therefore, for different cancers, it is necessary to make a different selection of viral types and design different modification schemes to construct OVs to achieve accurate tumor targeting and therapeutic effectiveness. The tumor microenvironment (TME) status is also an important aspect to be considered in virus selection and modification. Solid tumor cells often secrete cytokines such as interleukin (IL)−10, transforming growth factor β (TGF-β), and chemokines, which inhibit cellular immunity and recruit immunosuppressive cells, so that tumor cells can escape immune surveillance. Immune checkpoint receptor molecules (such as PD-1, CTLA-4, TIM-3, and LAG-3, etc.) are highly expressed in the infiltrated lymphocytes in TME, which contributes to the formation of an immunosuppressive TME [11]. Abnormal blood supply, low pH microenvironment, and anaerobic glucose metabolism of cancers can inhibit cancer cell apoptosis, promote angiogenesis and upregulate tumor growth factors, making cancer cells more resistant to radiotherapy, chemotherapy and immunotherapy [12]. Hence, it is necessary to consider how to improve the design of OVs, to increase their spread and persistence in TME, as well as gene expression such as the immune regulatory factors or anticancer factors, so that the purpose of treatment has been perfectly reflected.

Adenovirus

Adenovirus is a linear, 36-kb double-stranded DNA virus. Adenovirus enters cells through endocytosis or receptor-mediated mode and then releases genomic DNA to transfer to the nucleus for replication. Its DNA is always kept outside the chromosome and does not integrated into the host cell genome, which is one of the safety characteristics. The optimization design of OAVs mainly focuses on their oncolytic properties and safety, and there are four main strategies to modify targeting mechanism for adenovirus: (1) The E1B-55KD gene, which is necessary for its replication in normal cells but not necessary in tumor cells, is knocked out, and the representative products include ONYX-015 [6] and H101 [3]. (2) The expression of specific genes necessary for viral replication in cells is placed under the control of tumor-specific promoters. The representative products include OBP-301 (telomerase promoter) [13] and CG0070 (E2F-1 promoter) [14]. (3) Adenovirus capsid protein was modified to change the viral affinity and increase the efficiency and specificity of viral infection. For example, OBP-405 was developed on the basis of OBP-301 to infect tumor cells without coxsackievirus-adenovirus shared receptor (CAR) [15]. In addition, the modification of OAVs also includes the viral fiber capsid protein is modified with the Arg-Gly-Asp (RGD) peptide or modified into a chimeric fiber with two serotypes of adenoviruses, which can solve the issue of low infection efficiency caused by low expression of CAR in cancer cells. For example, Ad3/Ad11p and Ad5/F35 chimeric OAVs [16, 17]. On the basis of RGD, E1a was further modified to form a Delta-24-RGD OAV. Deletion of 24 bp within E1a makes E1a protein lose function to bind Rb protein, so that Rb protein in cells infected with Delta-24 adenovirus was released from E1a-Rb complex to block E2F activity, which is one of mechanisms to enhance the anticancer activity of OAV [18]. The viral hexon capsid protein is modified into chimeric hexon with adenovirus serotype rare in nature, which can enable the virus to escape the neutralizing antibody, attenuate the inactivation effect of immunity on adenovirus, and also help to avoid the adsorption of liver cells [19]. (4) Adenovirus E3 region transcripts and translates multiple proteins. Although they are not necessary for viral replication, their main function is to destroy the immune defense mechanism of the host, thus influencing the oncolytic effect. For example, the gp-19KD protein can inhibit the expression of major histocompatibility complex (MHC) class I molecules and prevent the infected cells from being recognized by effector T cells [20]. Adenoviral death protein (ADP) can lyse the infected cells and release virions in the late stage of viral infection [21]. Most OAVs are completely deficient in the E3 region, which can increase the load capacity of the virus. It is obvious that the deletion of part of the E3 region is conducive to viral replication and long-term gene expression, whereas the retention of part of the E3 region including ADP is conducive to viral release and diffusion. Therefore, multiple modifications can improve the infection efficiency or targeted safety of OAVs and improve their therapeutic effect.

Herpes simplex virus (HSV)

HSV is a DNA double-stranded virus that is divided into types 1 and 2. The type commonly used for OVs is type 1 (HSV-1). HSV-1 has a 152 kb genome in size and encodes 84 proteins. HSV-1, with its natural neurotropic and highly effective ability to infect, has been widely used in cancer treatment and has become an effective activator of innate and adaptive immunity. The replicative oncolytic HSV-1 is obtained by mutating or deleting those genes that play a key role in viral replication in normal cells and are not needed in tumor cells, including genes such as thymidine kinase (TK), ICP34.5, ICP47, and ICP6 [22]. ICP34.5 is required for HSV-1 replication in nerve cells, and knockout of ICP34.5 can inhibit viral replication in nerve cells but ensure efficient viral replication in rapidly dividing tumor cells [23]. ICP6 is the large subunit of HSV-1 ribonucleotide reductase, which is required for viral DNA replication in normal cells but not in tumor cells. Deletion of the ICP6 gene significantly reduces viral replication in normal cells and does not influence viral replication in tumor cells [24]. ICP47 can inhibit antigen presentation by MHC-1, and the deletion of ICP47 can effectively activate host antitumor immune response [25]. The approved T-vec is an OV

with knockout of ICP34.5 and ICP47 of HSV-1 to prevent its replication in normal cells, but without any influence on its replication in tumor cells [26]. Another OV, HSV1716, is developed by deletion of double copies of ICP34.5, so that it cannot replicate in neurons and other resting cells, but it is very efficient in replicating and lysing tumor cells. In 1996, HSV1716 was first approved for clinical trials in Europe. Clinically, HSV1716 has been used in the treatment of glioblastoma multiforme, melanoma, and head and neck squamous cell carcinoma, with good tumor regression results and no obvious side effects [27]. Although most oncolytic HSVs have been developed with direct deletion of ICP34.5, one study suggested that in malignancies with residual type 1 interferon (IFN) signaling or underlying IFN-dependent antiviral status, retention of ICP34.5 may enhance viral replication and oncolytic effect. To solve this issue, based on the regulation mechanism of microRNA on gene expression, the cancer- or tissue-specific microRNA can be used to control the targeting replication of OVs by inserting microRNA complementary sequence into 3′ untranslated region (UTR) of viral replication gene [28]. Therefore, researchers proposed a solution by inserting a miRNA responsive target element (miR-T) into HSV to control the expression of ICP34.5 gene, which not only enhances viral replication capacity but also ensures its selective replication in cancer cells [29]. The microRNA genetic switch provides a potentially versatile mechanism for solving the leakage mechanism that inhibits viral replication in cancer cells as well as permits viral replication in normal cells.

Vaccinia virus (VV)

VV is a double-stranded DNA virus with a 190 kb genome in size. The genome of VV is very large and can be inserted with large fragments of transgenes. VV is engineered to be oncolytic by knocking out its TK gene. VV replication is related to the level of TK in cells. VV with TK knockout can only replicate in cancer cells but not in normal cells [30]. Oncolytic VV replicates rapidly; its genome is not integrated into the host cell chromosome and does not cause insertion mutagenicity. Because of its low oncolytic efficacy, enhancing its antitumor activity is the focus of research. Pexa-vec (JX-594) is an oncolytic VV that expresses granulocyte-macrophage colony-stimulating factor (GM-CSF), which can activate the systemic immune response and inhibit tumor cells [31]. Pexa-vec takes advantage of the unique characteristics of two morphologically distinct infectious forms, one form of unenveloped IMV (intracellular mature virus) and another form of enveloped EEV (extracellular enveloped virus), evading neutralizing antibodies in the blood with the extracellular envelope and allowing its simultaneous intravenous and intratumoral injection [32]. Pexa-Vec has been approved as an orphan drug for the treatment of liver cancer by the European Medicines Agency (EMA) and the FDA of the United States, and it is currently approved by the China Food & Drug Administration (CFDA) for the treatment of advanced liver cancer in Phase III clinical trial, but the trial was terminated in August 2019 because the study did not meet its primary endpoint of overall survival.

Newcastle disease virus (NDV)

NDV is a single-stranded negative-stranded RNA virus with a total length of 15 kb genome, encoding six structural proteins and at least two non-structural proteins. NDV is a natural oncolytic virus, and after selectively infecting tumor cells, NDV plays an oncolytic role and induces an immune response to enhance the cytotoxic killing effect on cancer cells [33]. Studies have found that it is difficult to obtain good specificity and therapeutic effect by relying solely on the natural oncolytic ability and anticancer activity of NDV. Therefore, it is also necessary to recombine and modify NDV to enhance its targeting and effectiveness against cancers. By establishing the viral reverse genetic system, the oncolytic activity of the manipulated virus can be further enhanced, and NDV can be used as the vector of gene therapy to load anticancer genes, thus effectively improving the anticancer effect. Bai et al. constructed a recombinant rNDV and uploaded with interleukin-2 (IL-2) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). The results showed that IL-2 and TRAIL expressed by rNDV-IL2-TRAIL could significantly improve the anticancer effect by inducing apoptosis [34]. Cuadrado-Castano et al. constructed a recombinant NDV (rNDV-B1/Fas) encoding human Fas gene. In vivo studies in the syngeneic mouse melanoma model showed the enhanced oncolytic properties of rNDV-B1/Fas with significant improvements in survival and tumor remission [35].

Reovirus

Reovirus is a kind of double-stranded RNA virus that exists in the respiratory and intestinal systems of mammals. It has no obvious pathogenic effect under normal conditions, but it has targeted damage to cells with an activated RAS pathway. Since the RAS pathway is activated in various cancer cells, reovirus becomes a natural OV [36]. Reolysin is an unmodified wild-type oncolytic reovirus that can be administered intravenously. In 2015, it was approved as an orphan drug by the FDA for the treatment of ovarian and pancreatic cancers as well as glioblastoma. In 2017, it received FDA fast-track designation for the treatment of metastatic breast cancer. Phase II data for the treatment of advanced metastatic breast cancer showed that the combination of Reolysin and Paclitaxel increased overall survival from 10.4 months to 17.4 months [37]. For patients with brain tumors, the blood-brain barrier may inhibit OV delivery; hence, almost all studies to date have used direct intratumoral injection of OVs, and few studies have used intravenous administration for brain tumors. Samson et al. achieved a breakthrough in treating recurrent high-grade gliomas and metastatic brain tumors with intravenous reovirus, demonstrating that reovirus can cross the blood-brain barrier to reach the tumor site, replicate, and kill tumor cells. Moreover, T cell infiltration is stimulated and induced to exert anti-tumor immunity. Reovirus can upregulate tumor PD-L1 expression through an IFN-mediated mechanism [38].

Coxsackie virus

Coxsackie virus is a common group of viruses that infect humans through the respiratory and digestive tracts. There are 30 serotypes, including 24 serotypes in group A (A1-A24) and 6 serotypes in group B (B1-B6). Cavatak is a natural OV based on coxsackie virus A21 (CVA21), which was approved as an orphan drug by the FDA in Dec 2005 for the treatment of advanced melanoma [39]. Cavatak can specifically bind cancer cells with high expression of intercellular adhesion molecule-1 (ICAM-1) protein and insert itself into cancer cells, replicate and lyse cancer cells [40]. Coxsackie virus B3 (CVB3) also has a natural tendency toward cancer cells, its replication and oncolytic capacity are enhanced by the overexpression of decay-accelerating factor (DAF; also known as CD55) and coxsackievirus-adenovirus receptor (CAR) in some cancer cells [41].

Measles virus (MV)

MV is a single-stranded nonsegmental negative-strand RNA virus, which can recognize and infect a variety of cancer cells expressing CD46 and nectin-4 [42]. MV has been demonstrated to have good oncolytic activity. MV-NIS is a recombinant oncolytic measles virus expressing the human thyroid sodium iodide transporter (NIS) protein. By identifying the receptor protein CD46, MV-NIS enters cells and drives intercellular fusion between the infected myeloma cells and the uninfected neighbor cells to form non-viable multinucleated syncytia. Myeloma cells overexpress CD46 and are therefore highly sensitive to the killing by MV-NIS. MV-NIS is currently in phase I/II clinical trials [43].

Poliovirus (PV)

PV is an unenveloped single-stranded RNA virus with natural neuroinvasiveness. It invades cells through the receptor CD155 (also known as Necl5) on the cell surface. Because CD155 is widely expressed in solid tumors such as glioma, PV has strong oncolytic ability [44]. The 5′ untranslated region (5′-UTR) of the PV genome contains the tissue-specific internal ribosomal entry sites (IRES). Gromeier et al. replaced the IRES of type 1 attenuated live PV vaccine (Sabin) with the IRES of human rhinovirus type 2 (HRV2) to increase the capacity of PV replication. The resulting recombinant nonpathogenic poliovirus-rhinovirus chimera virus (PVSRIPO) can selectively destroy glioma cells while eliminating neurotoxicity and preserving tumor-selective replication, at the same time, normal neurons are not affected [45]. In addition to killing tumor cells directly, PVSRIPO can also induce anti-tumor cytotoxic T cell response and play an antitumor immune role through the infection of human dendritic cells (DCs) and macrophages by producing persistent IFN release in TME [46]. The published results of the phase I clinical trial showed that treatment with PVSRIPO resulted in a long-lasting anti-tumor effect that significantly extended survival [44,47]. As a result, the FDA granted PVSRIPO breakthrough therapy designation to advance research on the treatment of glioblastoma alone or in combination with other therapies.

Other viruses

Vesicular stomatitis virus (VSV) is a nonpathogenic negative-strand RNA virus that relies on the deficiency of IFN signaling pathway in tumor cells and specifically targets tumor cells. VSV is a potential oncolytic virus. When IFN-β is inserted into VSV, the resulting oncolytic virus activates CD8+ T cells and NK cells to activate effective anticancer immune response. In lung cancer, combination of the JAK/STAT inhibitor Ruxolitinib and oncolytic VSV-IFNβ increased viral replication in cancer cells [48]. Maraba virus is a single-stranded RNA virus found in the Brazilian sand fly. Its genetically engineered oncolytic virus is called MG1. MG1-MAGEA3 vaccine is composed of Melanoma-associated tumor antigen A3 (MAGE-A3) expressed in MG1. MG1-MAGEA3 alone was insufficient to enable detectable adaptive immunity against tumor antigen in melanoma-bearing mice, but had a potent ability to boost pre-existing tumor-specific CD4+ and CD8+ T-cell immunity, and dramatically prolonged the median survival with complete remission in more than 20% animals [49]. M1 virus is a natural virus isolated from Hainan Island, China. The virus replicates in and kills cancer cells by taking advantage of the deficiency of the IFN signaling pathway in tumor cells [50].

Selection of anticancer genes armed in OVs

Although oncolytic virotherapy is a promising tumor treatment method, its anticancer effect is not ideal if it only depends on viral oncolytic ability. OVs can be used as vectors to express anticancer therapeutic genes or immunoregulatory genes, so that they can deliver genes, reshape TME, and help T cells recruit, transport and infiltrate to TME and kill cancer cells. Further study on the genetic characteristics of cancers has provided many target genes for cancer therapy.

Cytokines and chemokines

The immunosuppressive state of the TME is an important link that affects the efficacy of cancer therapy. Especially in the microenvironment of solid tumors, the number of antitumor immune cells, including T cells, natural killers (NKs), DCs and macrophages, is few, and their functions are impaired. Therefore, many OVs are designed to express cytokine and chemokine genes, and the expression of cytokines and chemokines can promote the presentation and recognition of tumor-associated antigens (TAAs), activate antigen-presenting cells (APCs),

increase CD4+ and CD8+ T cells, and induce the M1 phenotype polarization of tumor-associated macrophages (TAMs). This will overcome the immunosuppressive status of TME and produce an effective, persistent, and specific anti-tumor immune response [51,52].

The immunoregulatory factor GM-CSF is widely used in the design of OVs, and it has shown convincing results in preclinical tumor models or clinical trials. GM-CSF is a cytokine that strongly stimulates the proliferation, differentiation, activation, maturation, and chemotaxis of APCs such as macrophages and DCs. T-vec is an HSV-1 oncolytic virus that expresses GM-CSF, which has been demonstrated to stimulate the immune system to attack and destroy cancer cells, thus providing significant and lasting benefits for melanoma patients [53]. In a clinical trial of patients with stage III and early IV melanoma, 436 patients with aggressive malignant melanoma who could not be treated surgically were randomly selected to receive T-vec virotherapy, in which 163 patients survived an average of 41 months, whereas the average survival of 66 early-stage patients receiving control immunotherapy was only

21.5 months. Approximately 16.3% of patients treated with T-vec showed a lasting treatment response over 6 months, compared to only 2.1% of patients treated with control therapy [54]. A recombinant human GM-CSF oncolytic HSV-2 injection, named OH2, has the same modification strategy as T-vec [55]. The product OH2 has been approved by FDA for clinical trial for the treatment of advanced solid tumors in August 2021.

IFNs, one of the members of the multifunctional cytokine family, can be divided into type I (IFN-α, IFN-β) and type II (IFN-γ); they regulate humoral immunity and cellular immunity and enhance the immunity of macrophages and NK cells. In recent years, it has been found that IFNs can enhance antigen presentation, activate the anti-tumor immune response, and inhibit tumor proliferation, metastasis and angiogenesis, and they can function as innate and adaptive essential cytokines for preventing tumor development [56]. Therefore, scientists expressed IFNs using OVs as vectors to enhance immune response and anti-tumor function. It has shown certain efficacy in the treatment of various tumors, including hepatocellular carcinoma, pancreatic cancer, mesothelioma, myeloma, head and neck squamous cell carcinoma, and breast cancer [52]. Type 2 interferon IFN-γ is produced by stimulating T lymphocytes with specific antigens. It can not only activate effector T cells and improve the activity of NK cells, macrophages, and tumor-infiltrating lymphocytes (TILs), but it can also enhance the expression of surface antigens and antibodies of immune cells. In anti-PD-1 immune checkpoint therapy, full-fledged activation of antitumor T cells by anti-PD-1 is not direct, but rather requires communication between T cells and DCs and is licensed by IFN-γ and IL-12, suggesting the importance of IFN-γ and IL-12 in improving responses to checkpoint blockade [57]. IFN-γ is expressed and released in cancer cells as a signaling protein, which continues to stimulate TME cells to produce and release other cytokines such as IL-2, tumor necrosis factor (TNF), IFN-α, and CXCL9 to continuously amplify the immune effect [58].

Interleukins (ILs) are a group of cytokines produced by a variety of cells and play an important role in transmitting information, activating, and regulating immune cells, mediating the activation, proliferation, and differentiation of T and B cells in the inflammatory response. The most common ones used for OVs include IL-2, IL-7, IL-12, IL-15, IL-18, IL-23, and IL-24, among which IL-12 has more opportunities to load in OVs in tumor immunotherapy [52]. VG161 is a novel antitumor immunoenhanced oncolytic HSV-1 developed by Virogin Biotech Co., Ltd. (Shanghai, China), which simultaneously carries four genes of IL-12, IL-15/15RA (IL-15 and IL-15 receptor α subunit) and PD-L1 blocking peptide (PDL1B) to stimulate synergistic anti-tumor immune response [59]. VG161 is currently undergoing phase I clinical trials in Australia. T3011, an OV product for intratumoral injection by Immvira Pharma Co., Ltd. (Shenzhen, China), has successively launched phase I clinical trials in China and Australia, and it has also been approved by the FDA. T3011 is a recombinant oncolytic HSV that carries both IL-12 and PD-1 antibody genes. The OAV TILT-123 (Bavencio), produced by

TILT Biotherapeutics Ltd., is a dual-cytokine-armed human Ad5/3 chimeric adenovirus that can replicate only in human cancer cells with deficiency of retinoblastoma (Rb)/p16 pathway. TILT-123 carries IL-2 and TNFα for the treatment of patients with metastatic melanoma. It can be administered through systemic and local delivery [60]. Cont-VV, an oncolytic vaccinia virus encoding IL-7 and IL-12, was intratumorally injected into murine tumor-bearing immunocompetent mice and human tumor-bearing humanized mice, which activated the inflammatory immune response and resulted in complete tumor regression, both in treated tumors and untreated distant tumors. Combined treatment with Cont-VV and PD-1 antibody or CTLA4 antibody further increased the antitumor activity in tumor models unresponsive to each of the immunocheckpoint inhibitors (ICIs) [61]. This study suggested that oncolytic virotherapy with Cont-VV expressing IL-7 and IL-12 exerts antitumor activity through remodeling immune status to render tumors sensitive to immune checkpoint blockade.

Chemokines are also a large class of target genes that are selected to load in OVs. In some solid tumors, TME lacks immune cells and APCs; hence, it is necessary to recruit T cells, DCs, and NKs to enter TME for reconstructing immune function. OVs can lyse tumor cells and the lysed cells release TAAs, pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs). Moreover, some OVs can infect APCs, promote their functional maturation, and lead to type 1 IFN response [62]. These inflammatory and antigenic stimuli lead to a tumor-specific immune response that promotes the recruitment, transport, and infiltration of immune cells in TME. OVs induce inflammatory stimulators such as TNF and IL-1β, which also promote T cell infiltration. In addition, OVs can be designed to encode chemokines, providing a direct remedy for TME immunodeficiency. Loss or reduction of CCL5 in cancer cells leads to a significant decrease in CXCL9 expression in TAMs and DCs, which results in a gradual loss of CD8+ T cells in tumor tissues [58]. CCL5 is expressed by OVs and released into TME, which attracts immune cells to the tumor, and the cells are activated by tumor cell surface antigens. The immune cells release IFN-γ which leads to the secretion of CXCL9 by macrophages and DCs, CXCL9 further promotes immune cell infiltration into tumors [58,63]. Other optional chemokines such as CCL20, CCL21, CXCL4L1 and CXCL10 have been shown in tumor models or clinical trials to stimulate the proliferation and activation of T cells and NKs, enhance the production of IFN-γ, induce an anti-tumor inflammatory response, and improve the therapeutic activity of OVs.

Our group constructed an OAV, OncoViron, which was a triple-serotype chimeric OAV expressing two immunomodulatory cytokines (IL-12 and IFN-γ) and one chemokine (CCL5). OncoViron was demonstrated to specifically target a variety of solid tumor cells, mediate high expression of anticancer factors, and significantly inhibit cancer cell proliferation. On a variety of implanted solid tumor models in immunodeficient mice, immunocompetent mice, and humanized mice, OncoViron showed great antitumor effect on its own and in combination with PD-1 antibody and CAR-T cells by remodeling TME [64].

Immune costimulatory and coinhibitory molecules

In cancer immunotherapy, the activation of T cells is crucial to efficacy. T cell activation is regulated by the interaction of multiple cellular receptors (such as PD-1, CTLA-4, B7-H3, LAG-3 and TIM-3, etc.) through various regulatory pathways. In recent years, OVs are loaded with immune co-stimulating molecular genes or immune checkpoint molecular antibody genes, which has been used to enhance the activation of tumor-specific T cells, the release of antigen and the production of IFNs, and then promote the maturation of DCs and the presentation of tumor cell antigens, so that OVs have a stronger anti-tumor effect [65–67]. An OAV, Delta-24-RGDOX, expresses the costimulatory molecule OX40 ligand (OX40L), OX40L binds to a unique costimulatory molecule OX40 on T cells to promote the activation and proliferation of tumor-specific T cells [65]. Delta-24-RGDOX has effective antitumor

activity against glioma in the C57BL/6 mouse model. Delta-24-RGDOX combined with PD-L1 antibody can produce more effective and long-lasting anti-tumor-specific immunity [66]. Studies have investigated other costimulatory members of the TNF receptor superfamily such as CD30, CD40, and 4–1BB and have been demonstrated to play an immune-activating role in a variety of malignancies. A novel oncolytic HSV, NG34SCFVPD-1 expressing PD-1 antibody 9 (scFVPD-1) was demonstrated to show a persistent antitumor response in two preclinical glioblastoma mouse models. It also has anti-tumor memory [67].

Suicide genes

Suicide genes encode a class of enzymes that catalyze the transformation of nontoxic drug precursors into cytotoxic substances, resulting in the death of the receptor cells. Insertion of the suicide gene into OV genome can enhance the anticancer activity of the virus. For example, the HSV-TK gene can convert the antiviral drug Ganciclovir (GCV) into cytotoxic monophosphate GCV and then into triphosphate GCV, which acts as a chain terminator, interfering with DNA replication and leading to cell apoptosis. Similarly, the suicide gene that encodes cytosine deaminase converts the nontoxic 5-fluorocytosine (5-FC) into the highly toxic anti-cancer drug 5-fluorouracil (5-FU), which kills tumor cells. T601 (TG-6002) is a recombinant oncolytic vaccinia virus that knocks out the TK gene and the ribonucleotide reductase gene and expresses another suicide gene (prodrug convertase gene FCU1). FCU1 can catalyze the conversion of nontoxic 5-FC to toxic 5-FU and 5-fluorouridine monophosphate (5-FUMP). Hence, T601 has the dual therapeutic mechanisms of oncolysis and targeted chemotherapy. Compared to conventional chemotherapy, T601 is highly selective to tumor cells with fewer side effects. Data from preclinical studies showed that T601 had good antitumor activity against malignant solid tumors [68]. T601 is currently in Phase I/IIA recruitment. Other suicide genes include those encoding nitroreductase and cytochrome P450. The approach applying OVs with suicide genes takes an advantage of the "bystander" effect mediated by the toxic products passively diffusing to noninfected neighboring cancer cells, and of the synergy between chemotherapy and oncolytic virotherapy [69]. Importantly, the bystander effect may be significantly increased when the transgene-expressing cancer cells are lysed by OVs and the toxic products are released and diffused from the lysed cells. Notably, the virotherapy with suicide gene can produce a bystander effect, killing more nearby tumor cells by spreading toxic metabolites.

Other genes

Referring to tumor gene therapy strategies, OVs have also been used to express tumor suppressor genes or pro-apoptotic genes. The common tumor suppressor genes include P53, Phosphatase and tensin homolog deleted on chromosome 10 (PTEN), P16, and RB, and the apoptosis-inducing genes include Apoptin, Lactaptin, TRAIL, and mitochondria-derived activator of caspase (SMAC). Russell et al. constructed an oncolytic HSV (HSV-10) that expresses N-terminally extended isoform, Phosphatase and tensin homolog deleted on chromosome 10 alpha (PTENα), the expressed PTENα inhibits the PI3K/ Akt signaling pathway and enhance the inhibitory effect of OVs on tumor cells [70]. Similarly, OVs express various TAAs such as carcinoembryonic antigen (CEA) or prostatespecific antigen (PSA), which can be used as anticancer vaccines to induce effective and persistent systemic anti-tumor responses. Hutzler et al. developed a recombinant oncolytic MV, MVvac2-CLDN6, which encodes a tumor-associated antigen, namely tight linking protein Claudin 6. It has shown promising results in the treatment of highly aggressive melanoma and inhibition of lung metastasis of melanoma cells [71]. In addition, OVs express antiangiogenic genes, such as vascular endothelial growth factor (VEGF) receptor 1-Ig fusion protein, VEGF single-chain antibody, VEGF promoter targeted transcriptional inhibitor (KOX), endostatin, angiostatin, and canstatin.

Antitumor antibody genes can also be inserted into OV vectors, such as oncolytic HSV-1 armed with anti-PD-1 antibody, to enhance antitumor efficacy [72]. Recently, Bi-specific antibodies (bsAbs) targeting cell-surface molecule on T cells and TAA on malignant cells are potential in cancer immunotherapy; therefore, bsAbs are also called bi-specific T-cell engager (BiTE). A growing number of BiTEs are entering clinical trials [73,74]. Based on the advantages of BiTEs and OVs, arming OVs with BiTEs would maximize local concentrations of BiTE at the tumor site, redirect T cells to tumor cells and improve the antitumor activity of the whole treatment system. An OAV expressing BiTE that targeting to CD3 and EGFR, ICOVIR-15K-cBiTE, was demonstrated to mediate oncolysis, robust T cell activation and proliferation, and bystander cell-mediated cytotoxicity. Intratumoral injection of ICOVIR-15K-cBiTE increased the persistence and accumulation of TILs in lung and colorectal cancer xenograft models of hPBMC-humanized SCID mice [75, 76]. The first clinical trial of OV-BiTE, NG-641, has already been initiated in patients with metastatic or advanced epithelial tumors on January 2020 and will be terminated on December 2022 (NCT04053283). NG-641 is an oncolytic adenoviral vector that expresses fibroblast activation protein (FAP)-specific BiTE (FAP-TAc antibody) together with an immune enhancer module (CXCL9/CXCL10/IFNα) [1]. The results are eagerly awaited and may provide insights into safety and efficacy of OV-BiTE.

Therapeutic strategy of OVs

Oncolytic virotherapy has become a new trend for the development of anticancer drugs since the 1990s. OVs-mediated gene therapy has many advantages as aforementioned; however, OV replication may produce leakage in normal cells because of the complex mechanism of tumor-targeting regulation, especially some natural OVs whose replication does not create a wide enough security window between normal cells and cancer cells. Secondly, OVs have strong immunogenicity. When they enter blood, the body will produce different levels of immune response, and the viruses may be quickly eradicated, which also affects their efficacy. Therefore, further research and development of novel OVs are needed. In addition, it is very important to design reasonable treatment strategies according to the characteristics of different tumors and different viruses to improve efficacy. OVs alone can produce a certain curative effect, OVs combined with other cancer therapies can produce synergistic efficacy and show a strong therapeutic potential [10,77,78].

OVs combined with chemotherapy

OVs can be designed for the treatment of broad-spectrum cancers or for a special type of cancer according to the different mechanisms of targeting regulation. Some of the OVs can be combined with particular chemotherapeutic drugs and has a better effect in cancer treatment; the combined therapy not only synergizes but decreases the dosage of chemical drugs or shortens the course of treatment, thus reducing the side effects of drugs and the probability of drug resistance. In a phase I "3 + 3" trial $^{designed\ for\ nonmetastatic\ (T_2\text{-}T_3\ N_{0\text{--}2})\ triple\text{-}negative\ breast\ cancer}$ $^{(TNBC),\ T\text{-}vec}$ was used in neoadjuvant chemotherapy to evaluate two different dosing regimens. Chemotherapy drugs include paclitaxel, doxorubicin, and cyclophosphamide. Of the 9 patients enrolled in the trial, 5 patients achieved pathologic complete response (pCR), i.e., no invasive disease in breast or lymph node, and the remaining 4 patients had only small residual lesions, indicating that the combination of neoadjuvant chemotherapeutic agents with T-vec is feasible at FDA-approved doses. This combination has high activity in TNBC [79]. Another phase I clinical trial of OV, Reolysin, combined with paclitaxel/carboplatin chemotherapy in patients with metastatic or relapsed KRAS pathway activated non-small cell lung cancer (NSCLC), showed that the treatment is well tolerated by the patients and effectively controls the disease, and Reolysin did not increase the toxicity of

chemotherapeutic agents [80]. C-REV (Canerpaturev, formerly HF10), a naturally mutated oncolytic HSV-1, entered phase I trial combined with gemcitabine (GEM) and erlotinib in 12 patients with unresectable pancreatic cancer. Three of the nine participants who completed the trial were in partial remission (PR), four were stable, and two showed progression. Compared to other GEM combination trials, the survival of patients treated with C-REV in combination with chemotherapeutic agents was improved, indicating that the combination of C-REV and chemotherapy can yield a significant benefit [81]. Further mechanism studies suggested that GEM is an immunosuppressive difluoronucleoside antimetabolite anticancer drug, which can inhibit the production of neutralizing antibodies in the body, promote the replication and diffusion of OVs in tumors, and thus enhance the anticancer effect of OVs. However, C-REV can promote the accumulation of CD8+/PD-1 tumor-infiltrating T cells in the PD-L1-enriched TME [82]. In addition, OVs combined with chemotherapy drugs such as cyclophosphamide, temozolomide, mitoxantrone, and paclitaxel can improve the antitumor effect.

OVs combined with radiotherapy

Both radiotherapy and oncolytic virotherapy can greatly break TME in solid tumors. There is evidence that radiotherapy can increase adenovirus infectivity by upregulating CAR expression in colorectal (HCT116) and head and neck (SIHN-5B) cancer cells [83]. Once OV infects cancer cells, viral proteins interact with cellular proteins that are involved in response of the radiation-induced DNA damage and modulate the activity of signaling pathways [84]. For example, radiation activates the epidermal growth factor receptor (EGFR) pathway, and EGFR induces the activation of transcription factors through the PI3K/AKT and RAS/RAF/MEK/ERK pathways, and then enhance the expression of viral and cellular genes to promote viral replication in the irradiated cells [85]. The destruction of TME caused by radiation apparently contributes to the diffusion of viruses released from cancer cells [83]. Therefore, radiation-induced destruction of cancer cells and change in the TME may enhance the viral infection, replication, and diffusion, thus mediating enhanced viral oncolytic effect. The therapeutic activity of OVs is not limited to their oncolytic activity, but also includes interactions within the TME, blood vessels, and stromal immune cells. OVs-induced change of TME can enhance tumor susceptibility to radiation. The two therapeutic methods complement each other in mechanisms; the changes in the expression of some key genes and the activity of signaling pathways in cancer cells caused by both radiotherapy and virotherapy help each other to improve the therapeutic effects; thus, oncolytic virotherapy combined with radiotherapy can improve the curative efficacy and produce the overlay or synergy effect. A phase I trial of the genetically engineered oncolytic HSV-1, G207, was conducted in a cohort of pediatric and adolescent patients with biopsy-confirmed recurrent or progressive supratentorial brain tumors, the patients received G207 (10^7 or 10^8 plaque forming units) intratumoral injection. Some patients received 5 Gy radiotherapy within 24 h after administration of G207. Twelve patients treated with G207 were found to have no dose-limiting toxicity or serious adverse events, and 11 had a radiological, neuropathological, or clinical response; the median overall survival was 12.2 months (95%CI: 8.0 to 16.4). Till June 5, 2020, four of these 11 patients were still alive after 18 months of treatment with G207. G207 significantly increased the number of tumor-infiltrating lymphocytes. Intratumoral injection of G207 combined with radiotherapy has been shown to improve clinical response in children with recurrent or advanced high-grade glioma [86]. Vijayakumar et al. found that oncolytic NDV combined with radiotherapy can enhance the therapeutic effect of ICIs (anti-PD1 and anti-CTLA4) on mouse melanoma [87].

Radiotherapy is a common treatment for solid tumors. It is necessary to explore the treatment mode of OVs-assisted radiotherapy. Therefore, our group attempted to construct a synergistic therapy approach. We

developed a radiation-induced enhanced Survivin promoter-regulated OV using the radiosensitive CArG regulatory element of the Egr-L gene as an enhancer. In combination with radiation therapy, radiation can activate the CArG regulatory element installed in the Survivin promoter, multiply the activity of the promoter, further improve the viral replication activity and the expression level of the anticancer gene, and the combined therapy displays an ideal anticancer effect [88]. An open-label, one-arm phase II clinical trial was recently completed at the University of Tokyo in Japan, 13 adult patients with glioblastoma who had residual or recurrent tumors in the brain after being treated with radiation or temozolomide chemotherapy received repeated doses of the oncolytic HSV Delytact (TeserpatureV /G47Δ). An interim analysis showed that it met the primary endpoint of a one-year survival rate of 92.3%, with few adverse events. This compares with only a 15% one-year survival rate with standard treatment of radiotherapy and temozolomide chemotherapy after surgery [5]. The results highlighted the promise of Delytact as an effective immunotherapy option for aggressive central nervous system malignancies.

OVs combined with ICIs

The changes in some genes and proteins in tumor cells and TME, especially the establishment of an immunosuppressive state, not only contribute to the occurrence and development of cancer, but they can also serve as targets of immunotherapy and oncolytic virotherapy. Effective immunotherapy relies on the immune response in TME, mainly involving three aspects: high density of TILs can ensure a strong tumor immune killing effect; TILs effectively recognize tumor-specific antigens; the treatment can remove the inhibition of tumor immunotherapy in TME. Therefore, the combination of OVs and tumor targeting drugs can also assist each other in mechanism, jointly break the chains of the immunosuppressive microenvironment and improve the anti-cancer efficacy.

Some immunosuppressive signal receptors are overexpressed in cancer cells, and the number and activity of immune cells in TME are low. ICIs can inhibit the role of these signal receptors, break the immunosuppressive state of TME, and play an anti-tumor role. However, this process depends on the activation of T cells, which in turn depends on the presentation of TAAs by APCs and the secretion of cytokines. The absences of TILs and APCs in solid tumors greatly attenuate the efficacy of ICIs. Infection and replication of OVs in cancer cells directly lead to cell lysis. TAAs are released during rapid lysis of cancer cells, which triggers host anti-tumor immune response and chemotactic infiltration of natural immune cells (such as NKs, macrophages, and DCs). DCs present TAAs and release cytokines such as IFN-γ and IL-12 to activate killer T cells and inhibit regulatory T cells. The production of IFN-γ can further stimulate microenvironment cells to produce more cytokines such as IL-2, TNFs, IFN-α, and CXCL9, and chemotaxis more TILs and APCs to the TME [57,58]. Therefore, the combination of OVs and ICIs can achieve "dual" or even "multiple" anticancer effects.

Recently, several studies in clinical trials have demonstrated the efficacy of the combined therapy with OVs and ICIs. In September 2017, Ribas et al. reported the results of a phase Ib clinical trial of T-vec in melanoma patients. The combination of T-vec and PD-1 antibody was well tolerated with no dose-limiting toxicity. T-vec can effectively improve the efficacy of immunotherapy, significantly increasing CD8+ T cell infiltration, with an overall response rate (ORR) of 61.9% (CR 33%), much higher than the expected response rates (approximately 35−40%) for Keytruda or T-Vec used alone. Patients treated with the combined therapy increased CD8+ T cell infiltration, raised PD-L1 and IFN-γ expression levels, suggesting that oncolytic virotherapy can produce synergistic effects by altering TME when combined with ICIs [89]. In October 2017, data from a phase II clinical trial for the treatment of advanced melanoma with T-vec combined with CTLA-4 antibody (Ipilimumab) were reported. Among 198 patients enrolled, 98 received combination therapy and 100 received Ipilimumab monotherapy. The

results showed an overall response rate of 39% ($n = 38/98$) for patients in the combination group as compared to 18% ($n = 18/100$) for patients in the monotherapy group; 13 patients in the combination group had a complete response (CR), and 7 patients in the monotherapy group had CR [90]. Studies have shown that mismatch repair-deficiencies (dMMR) affect the clinical response to ICIs [91]. Cancer patients with dMMR showed higher response rate to PD-1 antibody. The relationship between dMMR and the efficacy of oncolytic virotherapy was also demonstrated. A dMMR CRC model of MC38 tumors in mice, which moderately responded to ICI therapy but did not maintain durable responses, was treated by combinations of mitomycin C (mito) with oncolytic HSV-1 (oHSV). The results demonstrated that the addition of mito + oHSV was successful in further sensitizing tumors to ICI therapy, resulting in durable responses in 55% of mice, and the therapeutic efficacy of combined therapy was dependent on the infiltration of activated type 1 conventional dendritic cells [92]. In April 2021, Zhang et al. conducted a multicenter clinical study in China and reported the results from an oncolytic HSV-2 (OH2), which was applied to the safety and tolerability of patients with advanced solid tumors and antitumor activity in phase I/II clinical trial, as a single drug or with antibody against PD-1 (HX008). The trial included 54 patients with advanced solid tumors who had failed to respond to standard treatment, including 40 patients treated with OH2 alone and 14 patients treated with OH2 combined with HX008. Four patients with mismatch repair-proficient metastatic advanced rectal cancer or metastatic esophageal cancer, two patients from the monotherapy group, and two from the combination group achieved immune partial responses (IPR). The response duration of the two patients receiving OH2 monotherapy were 11.25+ and 14.03+ months, respectively. Two patients in the combination group had response durations of 1.38+ and 2.56+ months, respectively. The CD3+ and CD8+ cell densities and PD-L1 expression in tumor tissues were significantly increased after treatment in the monotherapy OH2 group. The results suggested that intratumoral injection of OH2 is well tolerated and shows durable antitumor activity [93].

OVs combined with CAR-T therapy

Chimeric antigen receptor T cell (CAR-T) therapy is currently one of the hot spots in cancer immunotherapy, which has completely changed the treatment model of hematological malignancies, but there are still huge obstacles in the treatment of solid tumors, largely due to the limitations of the immune state of TME. OVs can reshape the immunosuppressive TME of solid tumors, changing "cold" to "hot" tumors, and creating a microenvironment more conducive to the activity of T cells. Many preclinical studies have shown that the combination of CAR-T with OVs can increase CAR-T cell transport and enhance antitumor activity. However, to date, no clinical data from CAR-T cells combined with OVs have been reported, and only one investigational clinical trial (NCT03740256) is ongoing [94].

Reviewing the results of preclinical studies will help us to understand the potential of OVs in combination with CAR-T therapy. There have been only a few reports on OVs combined with CAR-T studies. Nishio and Dotti combined OAV expressing CCL5 and IL-15 (Ad5Δ24.RANTES.IL-15) with CAR-T cells targeting ganglioside GD2 (GD2.CAR-T) to treat neuroblastoma. The results showed increased overall survival in tumor-bearing mice and an improved function and survival of CAR-T cells, suggesting that OV-expressed CCL5 and IL-15 enhanced the transport and persistence of CAR-T cells, thereby reinforcing the antitumor effect [95]. Similarly, OAD-TNFα-IL2, a recombinant OAV expressing TNF-α and IL-2, was used in combination with the mesothelin-redirected CAR-T cells to significantly promote tumor regression in mice with human pancreatic ductal adenocarcinoma and enhance the antitumor effect of CAR-T cells [96]. To overcome the limitation by the lack of both tumor-restricted and homogeneously expressed tumor antigens in solid tumors, Park et al. designed an oncolytic vaccinia virus (OV19t) that encoded a non-signaling truncated CD19 (CD19t) protein, OV19t was

used to deliver CD19t to solid tumor cells, so that the cancer cells displayed the expression of the novel tumor antigen CD19t on the cell surface, and then CD19-CAR-T cells could recognize and destroy cancer cells. Multiple mouse tumor models have shown a strong synergistic effect between OV19t and CD19-CAR-T, with CR in 60% of tumor-bearing mice, compared to only 22% of mice treated with OV19t alone [97]. Another strategy for combination therapy is to make CAR-T cells load and deliver OVs. This strategy addresses two major challenges facing oncolytic virotherapy and CAR-T therapy in solid tumors. On the one hand, OVs can use CAR-T cells as a barrier to the immune system. On the other hand, OVs penetrates into the tumor cells to replicate and lyse tumor cells and trigger an immune response that facilitates CAR-T cell attacks on surviving tumor cells. Intravenous administration of OV-loaded CAR-T cells showed good efficacy in mouse melanoma and glioma tumor models, with improved survival [98]. This study provides an innovative approach to the treatment of solid tumors with OVs in combination with CAR-T cells.

However, the combination strategy of Ovs and CAR-T therapy is not yielding results as expected. Recent studies have shown that OVs reshape TME, which is not only beneficial but harmful to CAR-T cell therapy. Evgin et al. discovered an unexpected antagonistic mechanism in the treatment of fully immunized mouse B16EGFRvIII tumor model using EGFRvIII third-generation CAR-T cells; infection of oncolytic VSVmIFNβ virus in tumors resulted in severe attrition to CAR-T cells. The degree of CAR-T cell loss was proportional to the concentration of type 1 IFN production in the tumor after viral infection, whereas CAR-T cells that did not express type 1 IFN receptor (IFNAR1) were insensitive to type 1 IFN and thus resistant to OVs-induced CAR-T loss is developed [99]. The findings revealed an unexpected mechanism of therapeutic interference and remind us to further investigate the interaction between CAR-T cells and OVs for optimizing combination therapy, such as the selection of CAR-T cells insensitive to IFN for combination therapy with OVs may be more appropriate.

Perspectives of oncolytic virotherapy

The development of OVs and the progress of oncolytic virotherapy in clinical trials provide more options for cancer therapy (Supplementary Table S1). However, the effective approaches of oncolytic virotherapy are mostly combination therapies, and the efficacy of OV alone is not stable, depending on the immune status of patients, type of tumors, selection of OVs, etc. To improve the response of patients to oncolytic virotherapy, it may be necessary to develop more customized OV varieties, including broad-spectrum OVs for most tumors, and personalized OVs for specific tumor types or for specific genetic characteristics of cancer cells. OV varieties can be accurately selected according to clinical conditions to develop personalized treatment strategies.

At present, an effective objective response was identified in patients administered with OVs through intratumoral injections, but not in those treated with OVs via intravenous injections. Intratumoral injection limits patients who cannot be administered locally selection for oncolytic virotherapy. Intravenous administration of OVs is too easy to be cleared by the host immune system; hence, there are very few varieties of OVs that can resist immune system interception. In addition, through intravenous administration, a certain dose of virus is immediately diluted by blood, and if a large increase in virus dose was used to seek effective outcome, which may cause safety problems. To solve these issues, the first is to further study the immune mechanism of the virus and develop OV varieties that can effectively resist the immune clearance. The second is in combination with clinical interventional therapy, so that patients who can do interventional therapy can choose oncolytic virotherapy. For tumor patients undergoing interventional therapy, although OVs are not directly injected into tumor tissue, they can be delivered into TME, which will achieve the best therapeutic effect for those kinds of OVs with good targeting selectivity and high replication ability in cancer cells.

The future exploration of OVs should focus on improving their effectiveness and safety, and the exploration of oncolytic virotherapy should focus on developing better strategy of drug delivery and combination therapy. Although we hope to develop OVs that can achieve the best efficacy alone without the combination of other therapy, the clinical treatment of cancer has entered the era of combination therapy, and very few patients receive only one therapy. Oncolytic virotherapy belongs to immunotherapy, and to gene therapy after loading therapeutic genes. When OVs loaded with tumor-specific antigen or tumor neoantigen genes, they have the concept of cancer vaccine. In short, with the progress of tumor immunology research, the development and application of OVs will not stop, and the future is promising.

Conclusions

In summary, oncolytic virotherapy has become an important branch of cancer immunotherapy. OVs can reshape TME through their oncolytic effect and the functions of their expressed anti-cancer factors, which not only improve OV replication ability and oncolytic efficacy, but also create favorable conditions for other therapeutic methods. Recently, there have been breakthroughs in preclinical and clinical trials of OV combination therapy, and extensive data have demonstrated that the oncolytic and immune-stimulating effects of OVs make them more effective in combination with other therapies. In this context, interest in OVs as a platform for combined anticancer therapies is growing. To better improve the security and effectiveness of OVs, on the one hand, it is necessary to continuously improve the systematic delivery capacity of OVs and increase the transmission and persistence of OVs in TME. On the other hand, in-depth study and clarification of the interactions and regulatory mechanisms among the host immune system, OVs and tumor cells can enable us to design more and better anti-cancer OVs. Weakening the antiviral mechanism of the body is also one of the approaches to improve the efficacy of oncolytic virotherapy. Although this is still a difficult problem, the modification of multiserotype chimeric viruses and the development of viral vectors for intravenous use have greatly advanced this problem [64]. It is believed that with the development of genetic technology, the targeting and specificity of OVs will be further improved, and the safety will be more guaranteed, which will certainly shed new light on the treatment of cancers.

Author contributions

Y. S. and L. Q. conceptualized the article; Y. S. and C. S. wrote the manuscript; L. Q. edited the manuscript. All authors approved the final manuscript for publication.

CRediT authorship contribution statement

Yinghan Su: Data curation, Writing – original draft, Writing – review & editing. **Changqing Su:** Conceptualization, Funding acquisition. **Lunxiu Qin:** Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

None declared.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2022.101530.

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