Poxvirus oncolytic virotherapy

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Lino E. Torres-Domínguez and Grant McFadden

Biodesign Center for Immunotherapy, Vaccines and Virotherapy, Arizona State University, Tempe, AZ, USA

ABSTRACT

Introduction: Over the last decade, advances in biological therapies have resulted in remarkable clinical responses for the treatment of some previously incurable cancers. Oncolytic virotherapy is one of these promising novel strategies for cancer therapy. A successful oncolytic virus promotes tumor cell oncology and elicits a robust long-term anti-tumor immunity.

Areas covered: Oncolytic poxviruses (Vaccinia virus and Myxoma virus) demonstrated encouraging results in multiple pre-clinical tumor models and some clinical trials for the treatment of various cancers. This review summarizes the advances made on poxvirus oncolytic virotherapy in the last five years.

Expert opinion: Many challenges remain in poxvirus oncolytic virotherapy. Two key goals to achieve are enhancing the efficiency of viral delivery to tumor sites and overcoming local tumor immune-evasion. Additional efforts are necessary to explore the best combination of virotherapy with standard available treatments, particularly immunotherapies. By addressing these issues, this new modality will continue to improve as an adjunct biotherapy to treat malignant diseases.

1. Introduction

The increased understanding of the critical role of the adaptive immune system in cancer development and progression has led to novel interventions using several immunotherapies, which could in theory overcome the disadvantages of classical anti-cancer therapies. Among these biological strategies, oncolytic virotherapy has emerged as a novel promising modality, with encouraging results in both preclinical models (Table 1) and clinical trials with human patients [1, 2]. The first oncolytic virus (OV) was approved in 2015 by the FDA to treat melanoma (T-Vec or Talimogene laherparepvec), a genetically modified Herpes virus, and currently many other candidates are being tested in clinical trials [3]. In China, the clinical use of H101 (Oncorine), a recombinant adenovirus, was approved in 2005 for the treatment of squamous cell carcinoma of head and neck [4].

OVs are capable of selectively replicating and killing transformed cells with a malignant phenotype, while sparing normal cells and tissues (Figure 1). The anticancer activities of OVs are derived from multiple cancer-killing mechanisms: the direct infection and oncology of tumor cells by the virus, the infection of tumor-associated endothelial cells that leads to vascular collapse, and the cytotoxicity mediated by the activation and recruitment of immune cells into the tumor microenvironment (TME) [5]. The immunogenic potential of OVs relies on their capability to selectively infect and lyse cancer cells, releasing tumor-associated antigens and triggering strong innate and adaptive immune responses against cancer-specific epitopes. Therefore, their ability to elicit robust anti-tumor immunity, particularly against neoantigens and cancer-specific markers, makes them a powerful immunotherapeutic agent to assist the treatment of cancer (Figure 1) [6, 7].

Among the different OVs that have been tested, several belong to the Poxviridae family. Poxviruses are double-stranded DNA viruses possessing a large and well-characterized genome (130–300 kb). Poxvirus particles are exceptionally large; their size is around 250 nm in diameter and 360 nm in length, characterized by having a round brick-shape. There are two different infectious particles: intracellular mature virus (IMV) and extracellular enveloped virus (EEV), they are structurally similar, however EEV carry an additional outer lipid membrane containing proteins absent from IMV [8]. The precise mechanism of poxvirus entry is not fully understood, but it has been proposed that a macropinocytosis process is involved. While poxviruses are capable of attaching to and entering a wide variety of mammalian cells, post entry factors (such as induced antiviral signaling pathways) are thought to define cellular and host tropism [9]. The poxvirus replication cycle takes place exclusively in the cytoplasm, which makes them attractive candidates as OVs, since there is no risk for viral DNA integration into the host genome [8, 9]. Poxviruses are also highly immunogenic, expressing multiple viral antigens, and exhibit a strong capacity to activate acquired antitumor immunity following replication within cancer tissues [10]. To date, six poxviruses from four different genera have been investigated as potential OV: Vaccinia virus (VV), Racoonpox virus and Cowpox virus (Orthopoxviruses), Myxoma virus (MYXV) (Leporipoxvirus), Yaba monkey tumor virus (Yatapoxvirus), and Squirrelpox virus (Unassigned) [11]. This
Multiple preclinical studies and clinical trials have validated the safety and efficacy of Poxviruses (VV and MYXV) as platforms for oncolytic virotherapy. The optimization of OV-delivery to the tumor site is critical to improve the success rate of virotherapy in clinical trials. The use of Poxviruses and other OVs to enhance the anti-tumor immune response is key for their success as oncotherapy. Engineering poxviruses with multiple therapeutic transgenes offers innumerable possibilities to design vectors with improved properties (immune stimulators, imaging, etc.). The most successful virotherapeutic approaches are those that act synergistically when combined with other anti-cancer therapies, particularly with immunotherapies. This box summarizes key points contained in the article.

### Table 1. Summary of Poxvirus virotherapy in preclinical animal models.

<table>
<thead>
<tr>
<th>Poxvirus</th>
<th>Strain</th>
<th>Type of cancer</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>VV</td>
<td>VVtk</td>
<td>Liver cancer</td>
<td>84</td>
</tr>
<tr>
<td>VV</td>
<td>Melanoma</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>VV</td>
<td>Mammary carcinoma</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>VV</td>
<td>Breast cancer</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>VV</td>
<td>Pancreatic cancer</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>GLV-2b372</td>
<td>Hepatocellular carcinoma</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>GLV-1h153</td>
<td>Triple-negative breast cancer</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>Metastatic liver cancer</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>oncoVV-TTL</td>
<td>Hepatocellular carcinoma</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>GLV-1h68</td>
<td>Melanoma</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>JX-594</td>
<td>Melanoma</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>vvDD-CXCL11</td>
<td>Pancreatic neuroendocrine tumors</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>vvDD-IL-15</td>
<td>Colon or ovarian cancer</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>vvDD</td>
<td>Peritoneal carcinomatosis</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Oncopox-Trail</td>
<td>Lung cancer</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>OWV-CXCR4-A-Fc</td>
<td>Neuroblastoma</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>MYXV</td>
<td>Ovarian cancer</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>MYXV</td>
<td>Gloma</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>MYXV</td>
<td>Ovarian cancer</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>MYXV</td>
<td>Gallbladder carcinoma</td>
<td>22, 79</td>
<td></td>
</tr>
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<td>MYXV</td>
<td>Lymphoma</td>
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</tr>
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<td>MYXV</td>
<td>Pancreatic cancer</td>
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<tr>
<td>MYXV</td>
<td>Multiple myeloma</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>MYXV-135KO-GFP</td>
<td>Multiple myeloma</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>MYXV-IL15</td>
<td>Melanoma</td>
<td>94</td>
<td></td>
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<tr>
<td>vMyx</td>
<td>Melanoma</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>vMyx</td>
<td>sPD1</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>vMyx-M011L-KO</td>
<td>Glioblastoma</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>vMyx-CRISPR/Cas9</td>
<td>Embryonal rhabdomyosarcoma</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>vMyx-ERP</td>
<td>Embryonal rhabdomyosarcoma</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>vMyx-Deep2</td>
<td>Canine tissue sarcoma</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>

GMCSF Granulocyte-macrophage colony-stimulating factor, MYXV, Myxoma virus; VV, Vaccinia virus.

The successful delivery of virions to the TME is among the key challenges faced by virotherapy in general (Figure 1). Most of the preclinical and clinical trials have exploited a systemic delivery of the purified virus, mainly by direct intravenous (IV) injection route [10]. This route in theory allows delivery to both the primary tumor and any overt or undiagnosed metastatic sites accessible to the circulatory system simultaneously. Data from clinical trials have shown that OVs can be delivered systemically safely and with limited toxicity, however the efficacy has not always met expectations [15]. A possible reason is that the injected free virions can be cleared very quickly from circulation through neutralizing antibodies, complement activation, antiviral cytokines, and tissue-resident macrophages, as well as nonspecific filtering uptake by other tissues such as the lung, liver and spleen. Thus, this limits the amount of OV that can effectively reach the TME [16]. Another route extensively used in preclinical models and clinical trials with VV is the intratumoural (IT) delivery route, which has proved to be well tolerated and safe in humans [10, 15]. Local delivery means less systemic toxicity while focusing the immune response on the TME and the affected lymph nodes. Nevertheless, this IT injection method is limited to easily reachable solid tumors and is more invasive for the patient. Other delivery strategies for specific types of cancer have been intraperitoneal (for peritoneal carcinomatosis) and intrapleural in 1977. Consequently, this virus has a well-established safety record in humans based on its extensive use [12]. While VV naturally displays preferential kinetics in cancer cells, it can productively infect a wide variety of animals and cell types, including non-dividing cells [10]. Hence, only genetically modified VV constructs selectively target tumor cells. The extensive success of VV as an OV in multiple preclinical models (Table 1) has led to the translation to clinic (Table 2) [10, 13]. Currently, there are three oncolytic VV variants being tested in clinical trials, with promising outcomes for the treatment of several malignant diseases [10, 11].

MYXV has a natural narrow host range within the lagomorphs and is only pathogenic for European rabbits (Oryctolagus cuniculus). This restrictive tropism in nature makes it a very safe agent for oncotherapy [9]. Although MYXV is nonpathogenic to humans and mice, the virus exhibits natural tropism for a wide spectrum of human cancers [14]. The selective replication in human tumor cells is based on multiple dysregulated intracellular pathways found in transformed cells. These antiviral pathways, on the other hand, effectively abort MYXV replication in most normal primary somatic human and mouse cells. Oncolytic MYXVs have been tested in a wide variety of animal tumor models, proving its effectiveness as virotherapy against many types of cancers [14].

The topic of poxvirus oncolytic virotherapy has been reviewed before [10, 11, 14–16]. Here, we intend to update the progress made in the last five years and to discuss the current challenges the field still faces.

2. Virus delivery: systemic vs intratumoral

2.1. Vaccinia virus

The successful delivery of virions to the TME is among the key challenges faced by virotherapy in general (Figure 1). Most of the preclinical and clinical trials have exploited a systemic delivery of the purified virus, mainly by direct intravenous (IV) injection route [10]. This route in theory allows delivery to both the primary tumor and any overt or undiagnosed metastatic sites accessible to the circulatory system simultaneously. Data from clinical trials have shown that OVs can be delivered systemically safely and with limited toxicity, however the efficacy has not always met expectations [15]. A possible reason is that the injected free virions can be cleared very quickly from circulation through neutralizing antibodies, complement activation, antiviral cytokines, and tissue-resident macrophages, as well as nonspecific filtering uptake by other tissues such as the lung, liver and spleen. Thus, this limits the amount of OV that can effectively reach the TME [16]. Another route extensively used in preclinical models and clinical trials with VV is the intratumoural (IT) delivery route, which has proved to be well tolerated and safe in humans [10, 15]. Local delivery means less systemic toxicity while focusing the immune response on the TME and the affected lymph nodes. Nevertheless, this IT injection method is limited to easily reachable solid tumors and is more invasive for the patient. Other delivery strategies for specific types of cancer have been intraperitoneal (for peritoneal carcinomatosis) and intrapleural
Both strategies were safe for these malignant diseases [15].

To address these delivery limitations in preclinical and clinical trial, original methods are being explored to increase the efficacy of VV delivery to the tumor bed. A study reported that the use of silk-elastin-like protein polymer matrix for intraoperative VV delivery retards the degradation of infectious viral particles, facilitates sustained viral delivery and transgene expression, and improves tumor control. Such optimization of methods of OVs delivery may enhance therapeutic outcomes [17]. Badrinath et al. used VV coated with poly lactic-co-glycolic acid nanofiber for tumor delivery in a colon carcinoma mouse model and confirmed the therapeutic efficacy of this approach [18]. Although in the past some

Table 2. Summary of Vaccinia virotherapy in clinical trials.

<table>
<thead>
<tr>
<th>Poxvirus</th>
<th>Strain</th>
<th>Type of cancer</th>
<th>Phase</th>
<th>Status</th>
<th>Sponsor</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>VV Wyeth</td>
<td>Pexa-Vec (JX-594)</td>
<td>Hepatocellular Carcinoma</td>
<td>Phase III</td>
<td>Recruiting</td>
<td>Sillajen</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hepatocellular carcinoma</td>
<td>Phase I/II</td>
<td>Completed</td>
<td>Sillajen</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colorectal Cancer</td>
<td>Phase I/II</td>
<td>Recruiting</td>
<td>Sillajen</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colorectal Adenocarcinoma</td>
<td>Phase I/II</td>
<td>Recruiting</td>
<td>Institut Bergonie</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soft-tissue Sarcoma</td>
<td>Phase Ib</td>
<td>Recruiting</td>
<td>Sillajen</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast Cancer</td>
<td>Phase I</td>
<td>Recruiting</td>
<td>Sillajen</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal Cell Carcinoma</td>
<td>Phase I</td>
<td>Recruiting</td>
<td>Centre Leon Berard</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melanoma</td>
<td>Phase I</td>
<td>Recruiting</td>
<td>Sillajen</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metastatic Tumor</td>
<td>Phase I</td>
<td>Recruiting</td>
<td>Centre Leon Berard</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Advanced Tumor</td>
<td>Phase I</td>
<td>Recruiting</td>
<td>Centre Leon Berard</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colorectal cancer</td>
<td>Phase I</td>
<td>Completed</td>
<td>Sillajen</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soft tissues in pediatric patients</td>
<td>Phase I</td>
<td>Completed</td>
<td>Sillajen</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Neuroblastoma, Rhabdomyosarcoma, Lymphoma)</td>
<td>Phase I</td>
<td>Completed</td>
<td>Sillajen</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Melanoma, Lung Cancer</td>
<td>Phase I</td>
<td>Completed</td>
<td>Sillajen</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal Cell Carcinoma</td>
<td>Phase I</td>
<td>Completed</td>
<td>Sillajen</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Squamous Cell Carcinoma of the Head and Neck</td>
<td>Phase I</td>
<td>Completed</td>
<td>Sillajen</td>
<td>15</td>
</tr>
<tr>
<td>VV Lister</td>
<td>GL-ONC1</td>
<td>Ovarian Cancer</td>
<td>Phase I/II</td>
<td>Recruiting</td>
<td>Genelux</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fallopian Tube Cancer</td>
<td>Phase I</td>
<td>Active, no Recruiting</td>
<td>Genelux</td>
<td>15</td>
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<td></td>
<td></td>
<td>Lung Cancer</td>
<td>Phase I</td>
<td>Completed</td>
<td>Genelux</td>
<td>62</td>
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<tr>
<td></td>
<td></td>
<td>Advanced head and neck carcinoma</td>
<td>Phase I</td>
<td>Completed</td>
<td>Genelux</td>
<td>61</td>
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<tr>
<td></td>
<td></td>
<td>Peritoneal Carcinomatosis</td>
<td>Phase I</td>
<td>Completed</td>
<td>Genelux</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Advanced Cancers (Solid Tumors)</td>
<td>Phase I</td>
<td>Completed</td>
<td>Genelux</td>
<td>15</td>
</tr>
<tr>
<td>VV WR</td>
<td>vvDD-CDSR</td>
<td>Solid tumors</td>
<td>Phase I</td>
<td>Completed</td>
<td>University Pittsburgh</td>
<td>26,63</td>
</tr>
</tbody>
</table>

MYXV, Myxoma virus; VV, Vaccinia virus
preclinical studies tested the possibility of exploiting carrier cells for viral delivery of VV vectors, none of these studies have been pursued or translated to the clinic [19].

2.2. Myxoma virus

The intracranial injection of MYXV in either immunocompetent or nude mice was nontoxic, demonstrating the safety of this vector even in immunocompromised animals [10,14]. On the other hand, Lilly et al. reported that unfractionated leukocyte populations from bone marrow (BM) transplant samples, and particularly shown for purified T cells and neutrophils, could act as carrier cells for MYXV to cancer cells located in the bone marrow or spleen. Thus, ex vivo MYXV-treated allogeneic BM transplantation efficiently ablated pre-seeded residual multiple myeloma (MM) in an immunocompetent murine model [20]. This novel leukocyte delivery approach has the potential to circumvent the typical problems associated with the systemic delivery of OVs [20]. Human T cells can also be armed ex vivo with MYXV and then can transfer MYXV to MM cells via cell-cell contact in vitro [21]. A similar strategy using BM-derived stem cells (BMSCs) as MYXV carriers was explored in a gallbladder carcinoma murine model [22]. Significantly, IV injection of MYXV-infected BMSCs dramatically improves the oncolytic effect of MYXV alone, almost at the same level as intratumoral (IT) injection [22]. In addition, administration of adipose-derived mesenchymal stem cells pre-loaded with MYXV led to long-term survival of mice bearing gliomas, indicating that those cells may also be suitable for use as MYXV-delivery vehicles to tumor sites [23].

3. Oncotropism: preferential infection of cancer cells

3.1. Vaccinia virus

To target specifically tumor cells without infecting healthy tissues is a critical aspect for the development of all OVs (Figure 1). Several VVs possess inherent affinity for cancer cells, and additional genetic modifications have been introduced to further promote cancer cell specificity. The mutation of thymidine kinase gene (TK) from the VV genome is the most common mutation for achieving more selective tumor cell restriction [24]. TK is involved in nucleotide biosynthesis pathways and the deletion of this gene limits viral replication to cells with high levels of TK activity and high intracellular nucleotide pools, typically seen in most cancer cells. In fact, the three VV candidates currently in clinical trials were all engineered by deletion of the viral TK gene, which engenders more selective infection of cancer cells [19]. The same strategy is being exploited for other OVs in preclinical studies [25]. Other VV genes related to nucleotide metabolism might be candidates to further improve viral restriction to cancerous cells [19]. Vaccinia virus WR ‘double-deleted’ virus (vvDD) combines a TK deletion with a mutation in the VV growth factor (VGF) gene: this vector showed little ability to replicate outside of tumors and exhibits increased cancer cell selectivity compared to TK− mutants in vitro [26]. Genetically engineered vvDD with a mutated A34R gene produces more total progeny virus and EEV, and can thus more effectively evade neutralization from poxvirus antibodies, increasing virus spread and is highly cytotoxic to cancer cells [27].

Recently, it has been proposed that the deletion of two virus-encoded decapping enzymes (D9 and D10) could generate an effective oncolytic VV. D9 and D10 remove protective cap structures from mRNA 5′-termini, accelerates mRNA decay and limits activation of host cell antiviral defenses. Therefore, these VV mutants are highly attenuated, fail to counter cellular double-stranded RNA-responsive innate immune effectors, and thus hyperactivate the host anti-viral enzyme PKR in non-tumorigenic primary cells compared to wild-type virus. In addition, they displayed anti-tumor activity against syngeneic mouse tumors of different genetic backgrounds [28].

3.2. Myxoma virus

MYXV has a strict natural tropism for rabbits and hares and is totally non-pathogenic for humans and other mammals; however, the virus is able to productively infect a wide variety of human and murine cancer types [14]. In an attempt to further attenuate MYXV to make it safe for all potential vertebrate hosts, several immunomodulatory proteins have been deleted from the viral genome. Some of the resultant MYXV mutants were host-range restricted even in rabbits, but still retained the capability to infect and kill cancer cells in vitro and in vivo. In particular, the recombinant M135R-knockout MYXV was more attenuated than wild-type MYXV in rabbits [29]. For example, the M135-knockout MYXV is fully oncolytic against human cancer cells yet completely nonpathogenic for all known vertebrate hosts [14].

The selective replication of MYXV in human tumor cells is based on multiple dysregulated intracellular pathways commonly found in transformed cancer cells, for example the failure to induce type I interferons (IFN) and/or tumor necrosis factor (TNF) antiviral responses or upregulation of the cellular serine/threonine kinase (Akt), all of which support MYXV permissiveness [10]. Indeed, the screening of human cancers for the downregulation of IFN/TNF response pathways or the upregulation of cellular Akt activation might prove to be useful biomarkers for future MYXV virotherapy in patients. Recently, several eukaryotic dead-box RNA helicases that regulate cellular tropism of MYXV in human cancer cells were identified. The modulation of these self-defense host helicases in future studies may improve the capacity of MYXV to infect an even broader spectrum of tumor cells [30].

F11L is a VV gene product that promotes virus exit and rapid spread by inhibiting Rho signaling. When the VV F11L gene was inserted in a recombinant MYXV, it considerably improved the cell-cell spread of the virus in natural host cells and replicated to higher levels in various human cancer cells [31].

4. Oncolyis: killing of poxvirus-infected cancer cells

4.1. Vaccinia virus

Although most cancer cells have acquired the ability to evade apoptosis and other programmed cell death pathways, OVs can often induce in virus-infected cancer cells an apoptosis-like death or other types of virus-mediated cell death such as:
necrosis, senescence and/or autophagy [5]. Traditionally, one of the main aims in the oncolytic virotherapy field is to improve and optimize the cancer cell killing capacity of the virus. In recent years, however, the tendency is to focus not only on increasing cytotoxicity but also on the release of cellular tumor-associated antigens, neoantigens and furthermore the induction of immunogenic cell death (ICD) to promote acquired antitumor immune responses (Figure 1). The precise type of virus-induced cancer cell death is thought to be critical in order to prime a robust antitumor immunity [5].

VV vectors have been armed with apoptosis-inducing proteins like apoptin (VV-GMCSF-Apo) or lactaptin (VV-GMCSF-Lact). Both of these recombinant VV vectors activated a set of critical cellular apoptosis markers in virus-infected cells, proving that the apoptosis-inducing proteins expressed from the VV genome can serve as an efficient tool for altering the death pathway of infected tumor cells. Nevertheless, these VV-expressing apoptotic proteins were not better at inducing classic ICD markers in infected cancer cells than the parental virus [32,33]. On the other hand, VV JX-594-GMCSF and TG6002 (genetically modified to permit transforming 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU)) showed promising results to partially induce ICD markers and also to stimulate dendritic cells (DCs) as a central part of the adaptive immune system in a human melanoma model [34]. These results support the need to search for better alternatives to increase the capacity of all OVs to induce ICD in target cancer cells, in order to optimally promote a robust anti-tumoral immune response.

By using a directed evolution selection process, by pooling different strains of VV, including Copenhagen, Western Reserve (WR) and Wyeth strains and the attenuated modified vaccinia virus Ankara (MVA), a new recombinant poxvirus was generated with increased oncolytic properties. Through selective pressure in cancer cells, the chimeric VV deVV5 possessed increased cancer cell killing capacity and tumor selectivity in vitro [35]. In another report, a VV (oncoVV) was engineered to express a gene encoding a Lectin protein which showed significant antitumor activity in a hepatocellular carcinoma mouse model [36]. These results support the need to search for better alternatives to increase the capacity of all OVs to induce ICD in target cancer cells, in order to optimally promote a robust anti-tumoral immune response.

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The construction of a novel oncolytic VV harboring the inhibitor of growth family member 4 gene (VV-IN4A) displayed greater cytotoxic efficiency, and induced cell apoptosis in pancreatic cancer cell lines, in vitro and in vivo [37]. In addition, Hu et al. constructed a VV recombinant that expresses the human membrane-binding TNF-related apoptosis-inducing ligand (TRAIL), TRAIL protein mainly induced apoptosis and inhibited necrosis in the virus-infected cells. In vitro this vector displayed a more robust cytotoxic effect in a lung cancer cell line than parental virus while in vivo it exhibited enhanced anti-cancer effects in two transplanted tumor models of lung cancer [38].

4.2. Myxoma virus

In MM cell lines, MYXV infection initiates apoptosis through activation of the extrinsic initiator caspase-8, which appears to be independent of extrinsic death ligands and instead correlates with depletion of cellular inhibitors of apoptosis. Consequently, MYXV might eliminate human MM cells through a pathway unique to oncolytic poxviruses [39]. A pro-apoptotic MYXV was constructed, by deletion of M011L, an anti-apoptotic viral protein. vMyx-M011L-KO induced apoptosis in patient-derived brain tumor initiating cells and in vivo it significantly prolonged survival in an immunocompetent glioma mouse model, making this proapoptotic OV a strong candidate for clinical translation [40].

5. Immune-stimulation: triggering anti-tumor immune responses

5.1. Vaccinia virus

Historically, the concept of oncolytic virotherapy implied a virus-mediated lysis of the tumor cells, but as multiple evidences revealed the significant immunotherapeutic potential of OVs, the field gradually shifted to a more virus-enhanced immunotherapeutic perspective [6]. Hence, harnessing the immunogenic capacity of OVs has broadly become a priority. Indeed, those OVs that exploit their immunotherapeutic abilities are the most effective in promoting long-term tumor regression [5,6,41]. In this direction, it was determined that a WR-Δ4 virus, with the combined deletion of four specific viral genes that act on metabolic, proliferation, and immune signaling pathways (A48R, B18R, C11R, and J2R), exhibits effective anti-tumor capabilities in vivo [42].

Most DNA-genome OVs, and some RNA-genome OVs, have been genetically engineered, by inserting a myriad of transgenes, to further improve their immunogenic and/or cancer cell killing properties (Figure 2) [6,13,41]. Accordingly, OVs have been armed with a wide variety of immunostimulatory genes, including cytokines, chemokines, co-stimulatory proteins, or receptor-inhibitors. To date, the most successful example is the insertion of granulocyte-macrophage colony-stimulating factor (GM-CSF), a molecule expressed by T-Vec (the only OV approved by FDA) [3] and also Pexa-vec, the most advanced VV strain in human clinical trials [24]. GM-CSF promotes recruitment and maturation of many immune cell types such as: DCs, macrophages and T cells [24,43]. A vvDD strain expressing a superagonist IL-15 protein elicited a potent adaptive antitumor immunity, which led to significant tumor regression and extended survival in murine models of colon and ovarian cancer [44]. The construction of a membrane-anchored IL-2 expressed from VV maintained this cytokine within the TME and reduced the systemic toxicity associated with the soluble ligand. VV-IL2-anchored effectively modified the cancer-immune set point and showed no additional toxic side effects in a variety of murine tumor models [45]. Several other cytokines have been inserted into VV and tested in preclinical models, such as: IFN-β and TNF-α but these have not yet been translated to the clinic, perhaps due to their capacity to trigger direct anti-viral effects [6]. Liu et al. reported that a VV armed with the chemokine CXCL11 (vvDD-CXCL11) enhanced therapeutic efficacy when it was delivered via IT injection in a murine AB12 mesothelioma model. Moreover, vvDD-CXCL11 induced a potent antitumor
immunity, due to the migration of higher numbers of tumor-specific T cells and reduction of suppressive chemokines in the TME [46]. Other chemokines that proved to be effective as anti-tumoral transgenes in VV are CCL5 and CCL19 [19].

A very innovative approach has been the insertion of innate immune adaptor genes into the VV genome, such as: TIR-domain-containing adapter-inducing IFNs (TRIF) and DNA-dependent activator of IFN-regulatory factors (DAI). The authors attempted to activate toll-like receptors (TLR3)-signaling pathways, which is associated with boosting a cytotoxic T lymphocyte (CTL) response. The results demonstrated that both transgenes significantly enhanced the immunotherapeutic activity of VV [47]. In a subsequent study, the treatment with DAI-armed VV resulted in significant reduction in the size of syngeneic melanoma tumors in mice, and the engineered virus triggered a long-lasting adaptive immunity against that cancer [48].

Arming OVs to counteract tumor-mediated immune evasion has been also explored, by inserting genes that block suppressive cytokines (eg TGF-β, IL-10) and regulatory cells (Tregs, myeloid-derived suppressor cells, MDSCs) in the tumor bed [6,19]. For instance, signals mediated by the chemokine CXCL12 and its receptor CXCR4 are involved in the progression of ovarian cancer through enhancement of an immunosuppressive state and tumor angiogenesis within the TME. Consequently, a VV that was armed with hyaluronan receptor CD44, a CXCR4 antagonist, was tested in a murine epithelial ovarian cancer model. Intraperitoneal delivery of VV-CD44 led to reduced metastatic spread of tumors and improved overall survival compared with oncolysis alone [49]. Subsequent studies also demonstrated that VV-CD44 inhibited metastatic growth of murine and human ovarian tumor variants that were resistant to paclitaxel and carboplatin. The tumors described were more prone to ICD and shown to have reduced immunosuppression in the TME [50].

In addition, Yu et al. constructed an OV expressing a Bi-specific T cell engager (BiTe), which are a fusion of two modified antibodies designed to transiently engage cytotoxic T-cells for contact-mediated lysis of selected target cells. VV encoding a BiTe against the tumor cell surface antigen EphA2 induced a strong T cell activation that resulted in potent antitumor activity in a lung cancer murine xenograft model [51]. The link of OVs with immunotherapy was shown also on a VV armed with an Ab blocking the murine programmed cell death protein 1 (mPD1), where the IT injection of VV-antiPD1 on a MCA 205 tumor murine model induced a massive infiltration of immune cells including activated lymphocytes, supporting the synergistic effect of this combination [52]. However, the strain of VV-GLV-1h376 encoding for a secreted human Cytotoxic T-lymphocyte-associated protein 4-blocking single-chain antibody (CTLA4 scAb) failed in increasing cytotoxic T cells at the tumor site, proving the complexity of the interaction between OVs, the immune system and the TME [53].

5.2. Myxoma virus

The co-expression of IL15 fused with the α subunit of IL15 receptor (IL15Ra) greatly enhances IL15 stability and
bioavailability. Hence, a recombinant MYXV (vMyx-IL15R-tdTr) was engineered to expresses an IL15Ra-IL15 fusion protein plus tdTomato red fluorescent reporter. In vivo experiments with immunocompetent C57BL/6 mice showed that subcutaneous B16-F10 tumors treated with vMyx-IL15Ra-tdTr exhibited delayed tumor growth and a significant survival benefit as compared with parental virus, and remarkably stimulates both innate and adaptive antitumor immune responses [54].

Bartee et al. inhibited the PD1/PDL1 pathway within the TME by designing a MYXV contract expressing a soluble ectodomain form of PD1 (sPD1) from virus-infected cells. This sPD1-expressing virus induced and maintained antitumor cytotoxic T-cell responses within directly treated tumors and proved safer and more effective than combination therapy using unarmed MYXV in combination with more traditional systemic aPD1 antibody. This study demonstrated that tumor-localized inhibition of the PD1/PDL1 pathway could significantly improve outcomes of oncolytic virotherapy with poxvirus vectors [55].

6. Poxvirus oncolytic virotherapy: preclinical models and human clinical trials

6.1. Vaccinia virus

The armed oncolytic vector VV Pexa-Vec (pexastimogene devacirepvec, JX-594) expressing GM-CSF, licensed originally by Jennerex Inc. and now developed by SillaJen, is the most clinically advanced VV with the potential to become the first OV of the Poxviridae family approved by regulatory agencies for treating human cancers [43]. Pexa-Vec is derived from the VV vaccine strain VV-Wyeth but with disruption of the viral TK gene and expression of the human GM-CSF and β-galactosidase transgenes under control of the synthetic early/late and p7.5 promoters, respectively. The mechanisms of action for Pexa-Vec, which has been shown in preclinical models and patients, includes selective tumor cell infection and lysis, antitumor immune response induction and tumor vascular disruption [24,43]. Pexa-Vec has been evaluated in 17 completed and ongoing clinical trials to date (Table 2). Over 300 patients have been treated by IV infusion and/or IT injection [15,43]. In general, Pexa-Vec treatment was well-tolerated up to a dose of $3 \times 10^7$ pfu/kg per patient, with transient flu-like symptoms (including fever and chills) being the most commonly reported adverse event. Pexa-Vec–related skin pustules were observed in a minority of patients following IV infusion [56]. Therefore, immunocompromised individuals and those with inflammatory skin conditions were excluded from subsequent enrollment on trials, since these individuals would have a higher risk of complication from VV vaccination [43].

Notably, Pexa-Vec was associated with dose-dependent delivery to multiple solid tumor types (including colorectal cancer, lung cancer, pancreatic cancer, and mesothelioma) and resulted in antitumor activity at high doses [57,58]. Some patients responded to treatment based on modified response evaluation criteria in solid tumors. JX-594 improved median survival in patients with local/metastatic HCC more strongly at high dose than at low dose (14.1 vs 6.7 months; p = 0.08) in a Phase II study [59]. However, in a Phase IIb clinical trial with HCC patients who failed sorafenib therapy, the virotherapy did not achieve the primary endpoint of prolonging overall survival in treated patients when compared to patients treated with standards therapies [43,60]. These observations support the current view that drug pre-treated (ie standard therapy failures) and therapy-induced immunocompromised patients respond less well to treatments with OVs in general, re-enforcing the concept that OV responses lead to long term tumor regression more frequently when the host immune system is as intact as possible.

Additional Phase II studies evaluating single-agent Pexa-Vec treatment administered by multiple IV infusions are currently undergoing in treatment-refractory patients with renal cell carcinoma and colorectal cancer [15]. There are also several Phase I/II trials currently ongoing on distinct types of solid cancers for evaluating the efficacy of Pexa-Vec in combination with checkpoint inhibitors. Moreover, a randomized Phase III study is undergoing, to compare treatment of Pexa-Vec followed by sorafenib vs treatment with sorafenib alone in patients with advanced hepatocellular carcinoma [15].

A second VV candidate in clinical trials is GL-ONC1, based on vaccinia strain Lister. This OV was generated by insertion of three expression cassettes (encoding Renilla luciferase-Aequorea green fluorescent protein fusion, beta-galactosidase, and beta-glucuronidase) replacing the genes F14.5L (virulence factor), J2R (encoding TK) and A56R (encoding hemagglutinin) in the viral genome [19]. GL-ONC1 has been evaluated in four completed Phase I/II human clinical trials with 89 cancer patients (Table 2) [15]. GL-ONC1 was tested against a wide range of cancer types, with various methods of administration (ie. IV or regionally), either as a single agent therapy or in combination with conventional therapy. In all of these clinical trials, GL-ONC1 was well-tolerated, without showing significant adverse side effects [61,62]. This OV achieved the expected goal of reaching, infecting and selectively killing tumor cells as well as triggering antitumoral immune responses. Furthermore, these Phase I studies indicate encouraging evidence of efficacy and clinical benefit on the treated patients [61,62].

The last VV-based vector to undergo clinical trials is vvDD, derived from vaccinia strain Western Reserve, the more virulent parent strain of VV in animal models, but which has been engineered for less pathogenicity and improved tumor selectivity through two targeted gene deletions (TK deletion and a mutation the VGF gene) [63]. Two Phase I clinical trials have been performed: an IT dose escalation clinical trial of vvDD in 16 patients with advanced solid tumors and a Phase I study of IV delivery on patients with advanced colorectal or other solid cancers [15]. In both trials the treatment with vvDD was well-tolerated in all patients and resulted in selective infection of injected and noninjected tumors and antitumor activity [26,63].

6.2. Myxoma virus

Oncolytic MYXV has been tested in a wide variety of animal models for cancers including: gallbladder cancer, melanoma, gliomas, medulloblastomas, acute myeloid leukemia, pancreatic cancer and hematological malignancies (Table 1) [10,14]. Overall, these preclinical studies have demonstrated the
effectiveness of MYXV as an oncolytic therapeutic agent against a variety of cancers for which there are no current therapies. Notably, MYXV has the unique ability of infecting and deleting contaminating human myeloma or leukemia cells from autologous bone marrow transplant samples ex vivo while at the same time sparring the normal CD34+ hematopoietic stem cells needed for immune reconstitution after immunoablative therapy. In fact, MYXV functionally eliminated MM-CD138+ cells from patient BM samples within 24 h of treatment ex vivo [64]. Thus, MYXV is currently being explored as an oncolytic therapy for clinical trials with patients receiving autologous BM transplants [14]. When investigated in a xenograft murine model of pre-implanted human multiple myeloma, the pre-infection of a human BM transplant sample with MYXV for just 1 h prior to transplantation greatly reduced the posttransplant mortality in immunodeficient NSG mice [65]. The control mice were subsequently shown to have died from acute graft-versus-host disease (GVHD), caused by donor T cells resident in the human BM transplant, whereas the ex vivo pretreatment of donor BM with MYXV prevented the disease. These observations were further extended by testing ex vivo MYXV virotherapy against residual murine MM in immunocompetent mice using an allogeneic mouse-mouse transplant model [20]. Importantly, despite the resistance to direct MYXV infection and oncolysis of this particular MM cell line in vitro, the ex vivo MYXV-armed allogeneic BM transplantation dramatically ablated pre-seeded residual MM in the BM and spleen in vivo [20]. Comparably, systemic delivery of MYXV in a syngeneic mouse model of MM significantly reduced tumor burden and prolonged survival time, while leaving the healthy BM niche intact [66]. Despite numerous preclinical studies supporting MYXV as an exceptionally safe and effective OV, it has not yet been translated to humans. Before being tested in clinical trials, some issues need to be addressed such as: good manufacturing practice (GMP) production of the virus, pharmacology/toxicology of the construct chosen for clinical development, FDA approval for the use of MYXV in humans, and potentially additional approval of leukocyte-mediated delivery of the virus.

7. Combination therapies: who does what with whom?

7.1. Vaccinia virus

In the oncology research field, the use of combination therapies, a modality that combines two or more distinct therapeutic agents, is becoming crucial in the design of more effective anti-cancer interventions. Particularly with VV, many studies have been conducted preclinically and in clinical trials to determine its efficacy when combined with other therapies (Table 3) [10,11,19].

In preclinical models, the combination of VV and radiotherapy strongly suppressed tumor growth and prolonged survival, as compared to the single treatments [67,68]. The authors suggested that the virally-mediated down-regulation of anti-apoptotic proteins may increase the sensitivity of cancer cells to the cytotoxic effects of ionizing radiation [67]. VV expressing the human sodium-iodide transporter protein (hNIS) have been found to have anti-tumor effect when combined with systemic radiation [13]. Chemotherapy is another classical therapy that has been extensively combined with oncolytic VV in preclinical models [10]. For example, the combination of VV oncolytic candidates with chemotherapeutic agents such as gemcitabine, paclitaxel, cisplatin, cyclophosphamide and sorafenib, significantly improved the tumor regression in different animal models [10,13,69,70]. Currently, there are several clinical trials ongoing that combine different VV candidates with chemotherapy [15]. A phase III clinical trial is in progress using the combination of Pexa-Vec followed by sorafenib vs treatment with sorafenib alone in patients with advanced hepatocellular carcinoma [15]. Also, a Phase I Trial of GL-ONC1 proved its safety when applied simultaneously with cisplatin and radiotherapy in patients with advanced head and neck carcinoma [62].

Immune checkpoint blockade has become one of the most successful of the novel immunotherapies and has demonstrated tremendous promise for the treatment of diverse types of cancer. Immune checkpoint inhibitors (ICIs), such as anti–programmed cell death 1 (PD-1) and anti–programmed cell death ligand 1 (PD-L1) antibodies, are now widely used in multiple anti-tumor interventions [71]. Several preclinical studies have investigated the combination of oncolytic VV and ICIs. Rojas et al. tested VV-WR in combination with anti-CTLA4 antibodies in mouse models. The authors concluded that the interaction between ICIs and VV is complex, with correct selection of viral strain, antibody and timing of the combination being critical for synergistic effects [72]. Furthermore, some combinations produced antagonistic effects and loss of therapeutic activity[72]. Another report showed that a PD-L1–inducing VV combined with PD-L1 blockade was able to reduce tumor burden and induce long-term survival in anti-PD-L1 resistant murine cancer models. This approach demonstrates how the two therapies can synergize in order to surpass the clinical limitations of each alone [73]. Similar results were obtained by Liu et al., who observed that VV attracted effector T cells and induced PD-L1 expression on both cancer and immune cells in the tumor, leading to more susceptible targets for anti-PD-L1 immunotherapy in colon and ovarian murine cancer models [74]. The positive combinations of OV and ICIs have been successful in murine models that used armed-VV expressing immunostimulatory transgenes like IL-15 [44] and IL-2 [45], by further eliciting a potent antitumor immunity. These results indicate that OV may promote the activation and migration of effector T cells to the TME, whereas the ICI may liberate the anti-tumoral immune response by relieving T cell inhibition. Currently, there are several clinical trials ongoing to test the combination of VV with ICIs (Table3) [15].

Combining two OV is an innovative alternative that remains to be more extensively investigated. For example, VV and vesicular stomatitis virus (VSV) synergistically enhanced each other, promoting better tumor penetration and prolonged survival in a cancer murine model [75].

7.2. Myxoma virus

Multiple interventions have preclinically tested the combination of MYXV with standard treatments, particularly, chemotherapeutic agents (Table 3). For instance, pretreatment
with replication-competent MYXV-sensitized tumor cells to subsequent cisplatin treatments and drastically improve survival in a murine syngeneic ovarian cancer [76]. Similar observations were made for models of disseminated pancreatic cancer, in which MYXV sensitized cancer cells to gemcitabine treatment, with the combination interventions showing higher efficacy than either of the monotherapies in promoting survival [77]. Additionally, Zemp et al. observed that MYXV in combination with rapamycin infects and kills brain tumor-initiating cells, significantly prolonging the survival of tumor-bearing mice [78]. The same synergistic effect of MYXV plus rapamycin was described in a gallbladder cancer-bearing mouse model [79]. Additionally, the application of cyclophosphamide increased MYXV infection of cancer cells in a glioma murine model, the combined approach being more effective than the monotherapies [80]. Interestingly, MYXV exhibited synergistic effects with multiple small molecule inhibitors against human brain tumor-initiating cells in vitro, supporting the fact that MYXV can potentiate the anti-cancer effects of many cancer drugs. Some of these compounds have not been previously shown to synergize with OVs in vitro [81].

Bartee et al. reported that the application of MYXV with anti-PD-1 Ab improved overall survival in a murine model of melanoma. Almost 30% of mice receiving combination therapy exhibited complete responses, whereas either monotherapy only delayed tumor growth. Note that the combined therapy produced an adverse side effect, as it led to an autoimmune associated disorder of resulting hair loss, indicating that caution needs to be exercised in controlling the known autoimmune complications of ICIs [55].

8. Conclusions

Poxviruses possess many advantages that make them excellent candidates for oncolytic virotherapy. For instance, with the notable exception of previously smallpox-vaccinated patients receiving VV therapy, poxviruses do not need to overcome any pre-existing anti-viral immunity that affects many other OVs, making them more suitable for systemic delivery, while others are highly vulnerable to host defense mechanisms like neutralizing antibodies. Moreover, the unique ability of MYXV to infect BM-derived leukocytes or PBMCs ex vivo and then co-migrate with diverse classes of leukocytes to tumor sites after infusion in vivo, make it an ideal candidate for the treatment of hematological cancers or metastatic cancers that lie within the patrolling reach of migratory leukocytes. Also, the extensive track record of vaccination makes VV a very safe agent for oncotherapy, MYXV’s highly restrictive natural rabbit-specific tropism in nature suggests a high biosafety profile in humans. Moreover, poxviral DNA cannot integrate into the host genome, which could happen for viruses that need to exploit the nucleus as part of their replication cycle. Poxviruses like VV and MYXV also replicate rapidly and efficiently in tumor cells, a key feature for oncotherapy use. They are highly immunogenic, being able to subvert the immune-evasion associated with the TME. Interestingly, when tested in primary human or mouse dendritic cells, MYXV was more stimulatory than VV at inducing dendritic cell activation associated with antigen presentation pathways [82,83], but the long history of VV as a vaccine platform indicates that this virus can also robustly induce long-term immunity against a wide variety of virus-encoded antigens. Finally, the large dsDNA genomes of poxviruses also allow them to be engineered with a high number of exogenous transgenes, being suitable to construct multiple armed OVs with enhanced immunostimulatory or other desired properties. Combined, all of these characteristics suggest that poxviruses like VV and MYXV should be included among the most optimal platforms for oncolytic virotherapy.

9. Expert opinion

Despite the progress obtained during the last few years in the field of oncolytic virotherapy, there are several key challenges that still need to be overcome for these OVs to succeed in the...
Clinic. One important goal is to develop more optimal and efficient delivery systems of OVs to the sites of disease. Many OVs have shown modest results when translated to the clinic, due to the limited ingress of virions into the tumor sites. Therefore, the search for novel and more effective routes for OV delivery is a priority, in order to increase their success as oncoltherapy. For example, the use of MYXV-armored carrier cells might considerably increase the success of virus delivery to the TME, by overcoming the natural physiological barriers that limit virus access to tumor beds in hard to reach niches and metastatic sites. Particularly for MYXV, due to his natural ability to be disseminated by virus-infected leukocytes in rabbits, it is an ideal candidate to be tested in this alternative systemic delivery method. Moreover, the approval of oncoltherapies using patient-modified leukocytes (CAR-T cells) lessen the risk for the exploitation of ex vivo MYXV-loaded leukocytes in clinical trials.

For poxviruses, their tumor cell oncotropism (artificially rendered by genetic engineering for VV, natural for the MYXV platform) makes them very safe therapeutic candidates, although in the case of VV some immunocompetent patients might be contra-indicated. For MYXV, properly designed human clinical trials must be conducted to investigate the biosafety and potential of this virus in cancer patients. There is also a need to identify patient biomarkers to predict which OV, not just poxviral candidates, will be appropriate to treat certain tumors in specific patients.

Although the most desirable feature for OVs may be the targeted death of the tumor cells, the concept of ICD as a desired end point of OV-infected cancer cells has changed this perspective. Thus, now researchers must focus not only on increasing the oncolytic capacity of the poxvirus vectors, but also on inducing ICD in the target cancer cells, thus promoting a more robust antitumor immune activation. The next generation of genetically modified poxvirus-based OVs (presumably achieved by mutations of viral genes and/or inserting exogenous transgenes) may be more efficient in inducing ICD in cells with a malignant phenotype.

Harnessing the immunotherapeutic potential of OVs will increase their potential impact because of the prospect of turning ICI-nonresponsive cancers into responsive ones. Notably, the possibility of bioengineering vectors to incorporate genetic modifications or transgenes to elicit a stronger and more sustained systemic anti-tumor immunity has become a milestone goal for the virotherapy field. Future generations of poxvirus backbones will be armed with multiple immunostimulatory factors, in some combination with inhibitors of immune-suppression or ICIs. Moreover, the restricted expression of these OV-engineered transgenes in the TME may reduce toxicity related risks. Nevertheless, further studies must investigate the transgenes that will best complement each other in a synergistic manner, as well as determine the best timing for the expression and release of these immune regulators. Importantly, the induction and kinetics of an early anti-viral response may also be manipulated in order to allow optimal viral replication and spread within the tumor.

Among OVs, the best candidates are those that display superior anti-tumor therapeutic effects when combined with other independent modalities or immunotherapy regimens (ICIs, CAR-T cells, etc.). Also, the sequential use of two immunologically distinct OVs may help to overcome the weaknesses of each the vectors. As discussed, combining two anti-cancer strategies can enhance efficacy compared to either monotherapy approach, if they work in a synergistic or even an additive manner. Further studies are necessary to define the optimal combinations with these anti-cancer therapies, which will serve to increase the positive effects of clinical outcomes. Without doubt, with so many OVs awaiting to be validated in clinical trials and multiple challenges to be addressed, this is an exciting time for oncolytic virotherapy.

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Declaration of interest

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Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.


**Paper reporting a novel method for OV-delivery**


**Describing the use of carrier cells for delivery of oncolytic MVV**


**First report of the insertion of adaptor proteins in an OV platform**


**First report of the insertion of adaptor proteins in an OV platform**


