



Universiteit  
Leiden

The Netherlands

## **Harnessing the immunostimulatory properties of oncolytic reovirus for anticancer immunotherapy**

Groeneveldt, P.C.

### **Citation**

Groeneveldt, P. C. (2023, November 23). *Harnessing the immunostimulatory properties of oncolytic reovirus for anticancer immunotherapy*. Retrieved from <https://hdl.handle.net/1887/3663612>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3663612>

**Note:** To cite this publication please use the final published version (if applicable).

PART

B

**The effect of preexisting immunity  
on reovirus therapy**



# CHAPTER 4

## Preexisting Immunity: Barrier or Bridge to Effective Oncolytic Virus Therapy?

**Christianne Groeneveldt<sup>1\*#</sup>**, Jasper van den Ende<sup>2\*</sup>, Nadine van Montfoort<sup>3</sup>

<sup>1</sup> Department of Medical Oncology, Oncode Institute, Leiden University Medical Center, 2333 ZA Leiden, The Netherlands

<sup>2</sup> Master Infection & Immunity, Utrecht University, 3584 CS Utrecht, The Netherlands

<sup>3</sup> Department of Gastroenterology and Hepatology, Leiden University Medical Center, 2333 ZA, Leiden, The Netherlands

\* These authors contributed equally

# Corresponding author

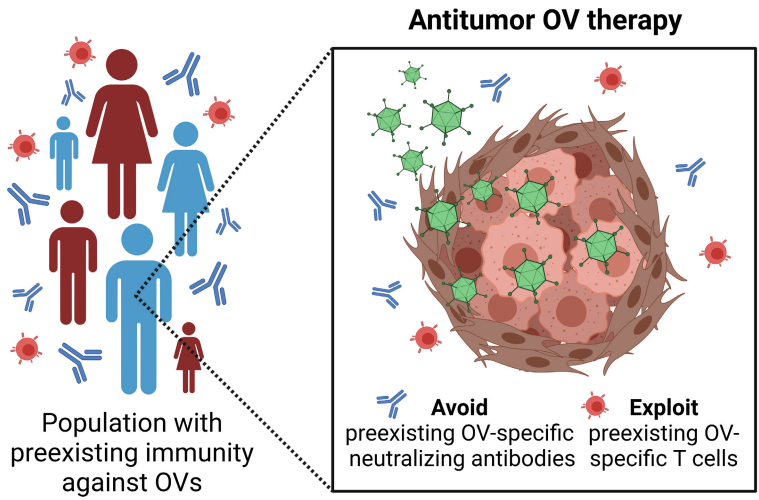
## ABSTRACT

Oncolytic viruses (OVs) represent a highly promising treatment strategy for a wide range of cancers, by mediating both the direct killing of tumor cells as well as mobilization of antitumor immune responses. As many OVs circulate in the human population, preexisting OV-specific immune responses are prevalent. Indeed, neutralizing antibodies (NABs) are abundantly present in the human population for commonly used OVs, such as Adenovirus type 5 (Ad5), Herpes Simplex Virus-1 (HSV-1), Vaccinia virus, Measles virus, and Reovirus. This review discusses (pre)clinical evidence regarding the effect of preexisting immunity against OVs on two distinct aspects of OV therapy; OV infection and spread, as well as the immune response induced upon OV therapy. Combined, this review provides evidence that consideration of preexisting immunity is crucial in realizing the full potential of the highly promising therapeutic implementation of OVs. Future investigation of current gaps in knowledge highlighted in this review should yield a more complete understanding of this topic, ultimately allowing for better and more personalized OV therapies.

### **List of Abbreviations**











Ad5; Adenovirus serotype 5	HSV-1; Herpes simplex virus type 1
ADE; antibody-dependent enhancement	ISG; interferon-stimulated gene
ATPP; Antibody-Targeted Pathogen-derived Peptides	MHC; major histocompatibility complex
BiTE; bispecific T-cell engager	NABs; neutralizing antibodies
CMV; Cytomegalovirus	NDV; Newcastle disease virus
CV-A21; Coxsackievirus A21	NOD; non-obese diabetic
DAMP; damage-associated molecular pattern	NOG; NOD.Cg-Prkdc <sup>scid</sup> Il2rg <sup>tm1Sug</sup> /Shijic
DC; dendritic cells	NSG; NOD.Cg-Prkdc <sup>scid</sup> Il2rg <sup>tm1Wjl</sup> /SzJ
EBV; Epstein-Barr virus	OV; oncolytic virus
EEVs; extracellular enveloped viruses	OVA; ovalbumin
FcγRs; Fc-gamma receptors	PAMP; pathogen-associated molecular pattern
FDA; Food and Drug Administration	TME; tumor microenvironment
HBsAg; Hepatitis B surface antigen	T-VEC; talimogene laherparepvec
	VSV; Vesicular Stomatitis Virus

# GRAPHICAL ABSTRACT



## INTRODUCTION

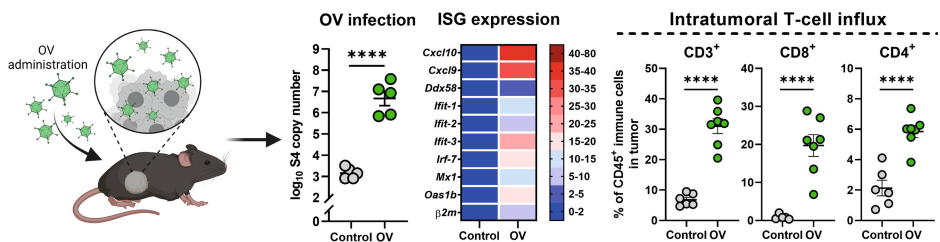
Oncolytic viruses (OVs) are increasingly being recognized as a promising therapeutic modality for the treatment of a variety of cancers (1,2). Selective replication of OVs in cancerous cells, which can either be a result of natural viral tropism or artificially achieved by genetic modification, makes them highly specific antitumor agents with minimal off-target effects. An overview of the most prominently investigated OVs is provided in **Figure 1**. Increasing interest in the clinical potential of OVs has been driven by the Food and Drug Administration (FDA) approval of the modified Herpes Simplex Virus type 1 (HSV-1) talimogene laherparepvec (T-VEC), which was shown to significantly improve survival in patients with late-stage melanoma (3-5). Currently, there is an immense pipeline of over 200 registered clinical trials investigating the therapeutic application of various OVs as single agents or as part of combination therapies (6).

										
OV platform	Adenovirus 5 (Ad5)	Herpes Simplex virus 1 (HSV-1)	Vaccinia virus	Measles virus	Reovirus	Vesicular Stomatitis virus (VSV)	Newcastle Disease virus (NDV)	Maraba virus	Coxsackievirus A21 (CV-A21)	Polio virus
Structure	naked	enveloped	enveloped	enveloped	naked	enveloped	enveloped	enveloped	naked	naked
Genome	dsDNA	dsDNA	dsDNA	(-)sense RNA	dsRNA	(-)sense RNA	(-)sense RNA	(-)sense RNA	(+)sense RNA	(+)sense RNA
Infects humans	✓	✓	✓	✓	✓	✗	✗	✗	✓	✓
Tumor-specific without genetic modifications	✗	✗	✗	✓	✓	✗	✓	✓	✓	✗
Transgene packaging possible	✓	✓	✓	✓	✗*	✓	✓	✓	✗	✗
Seroprevalence	✓	✓	✓	✓	✓	✗	✗	✗	✗	✓

**Figure 1. Properties of commonly investigated oncolytic virus (OV) platforms.** dsDNA indicates double-stranded DNA. Green checkmarks indicate that a characteristic does apply to the specific OV platform, red crosses indicate that it does not. The presence of seropositivity is derived from clinical trial data (serum samples measured before treatment), or population studies. References for general information about each OV and seropositivity data: Ad5 (7-10), HSV-1 (3,11-13), Vaccinia virus (14-17), Measles virus (18-22), Reovirus (23-29), VSV (30-32), NDV (33), Maraba virus (34), CV-A21 (35-37), Polio virus (38-40). \*For Reovirus, only packaging of very small transgenes is possible, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) (41).

Multiple mechanisms of action are known to contribute to the therapeutic efficacy of OVs, as was previously reviewed by us and others (42,43). Direct oncolysis is the result of viral repurposing of the infected cell for the production of viral genomic material and proteins, which eventually results in the release of progeny viral particles through cell lysis (44). Besides direct killing, there is accumulating evidence that shows that OVs can also stimulate strong immune-mediated antitumor effects (45). Local inflammation recruits immune cells to the tumor microenvironment (TME), where viral infection and killing of tumor cells result in the release of both pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) and type I interferons (46). These PAMPs and DAMPs mediate the potent activation of dendritic cells (DCs) for antigen presentation. In combination with high tumor antigen availability due to oncolysis, this constitutes

an OV-induced ‘perfect storm’ which establishes conditions uniquely favorable for efficient priming and subsequent influx of both virus- and tumor-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells (**Figure 2**). Recent investigations into the OV-mediated delivery of immune-stimulating transgenes into the TME, such as cytokines, costimulatory T-cell ligands, checkpoint inhibitors, or even tumor antigens, further illustrate the crucial importance of immunity in the context of OV therapy (47,48). Furthermore, OV therapy can promote the availability of tumor antigens. Most notably, OV-induced oncolysis of infected cells can result in the release of otherwise inaccessible tumor antigens, improving the immune response against cancer cells expressing these epitopes (49). Furthermore, OVs can be employed as so-called oncolytic vaccines, which encode or are coated with tumor antigens to steer the immune response toward antitumor specificity (50,51).



**Figure 2. Mechanism of action of immune-stimulatory effects of OVs in the tumor microenvironment.** OV administration leads to infection of tumor cells, which induces the upregulation of interferon-stimulated genes (ISGs) including T-cell attracting chemokines. The OV-induced expression of ISGs is followed by an increased influx of T cells into the tumor. Data is derived from studies where oncolytic reovirus is injected intratumorally in immunocompetent C57BL/6j mice bearing murine pancreatic KPC3 tumors (52,53). OV infection and ISG expression was determined by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and intratumoral T-cell influx was measured by flow cytometry.

Despite the immense therapeutic potential of OVs, some patients do not respond to OV therapy. One of the proposed limiting factors for effective OV therapy is the presence of preexisting immunity in patients (54). Therapeutic application necessitates the use of non-pathogenic OVs, but the fact that they are benign is often a result of the efficient immune response that is induced upon infection. Thus, previous exposure is likely to result in the presence of a potent preexisting immune response. In antiviral immune responses, circulating viral particles are recognized and subsequently neutralized by antibodies, whereas virus-infected cells are targeted by virus-specific cytotoxic CD8<sup>+</sup> T cells. Therefore, possible effects of preexisting immunity on OV infection and spread predominantly involve a preexisting humoral response. Indeed, assessment of OV-specific neutralizing antibodies (NAbs) in serum in both the general population and OV clinical trial cohorts, also termed seroprevalence, shows that preexisting immune responses are abundantly present. This is primarily the case for viruses that, besides their application as OVs, also circulate in the human population or are used as vectors for vaccination, such as Adenovirus serotype 5 (Ad5), HSV-1, or Vaccinia virus (**Figure 1**). Seroprevalence is much less common for OVs that mainly infect non-human hosts, such



as Vesicular Stomatitis Virus (VSV) or Newcastle Disease virus (NDV). So far, the general consensus has been that the presence of preexisting immunity decreases OV efficacy by enhancing viral clearance, thus limiting the window of therapeutic action. This has resulted in patient exclusion criteria based on the presence of neutralizing antibodies in some clinical trials, for example (NCT01227551) (55). However, emerging evidence suggests that OV-specific preexisting immunity might actually potentiate antitumor effects in some cases. Thus, a nuanced assessment of the effects of preexisting immunity in the context of OV therapy is warranted.

Here, we provide an overview of the currently available mechanistic insights regarding the effect of preexisting immunity in two distinct phases of OV therapy: 1) OV infection and spread upon administration, and 2) development of the OV therapy-induced immune response, while discussing the many variables that contribute to the effect of preexisting immunity in these phases. Furthermore, we discuss how preexisting immunity can be evaded or even utilized to enhance the therapeutic efficacy of OVs. By shining a light on the complex nature of preexisting immunity in the context of OV therapies, the collection of (pre)clinical data discussed here should prove instructive for future decisions regarding both fundamental investigation as well as the therapeutic application of OVs.

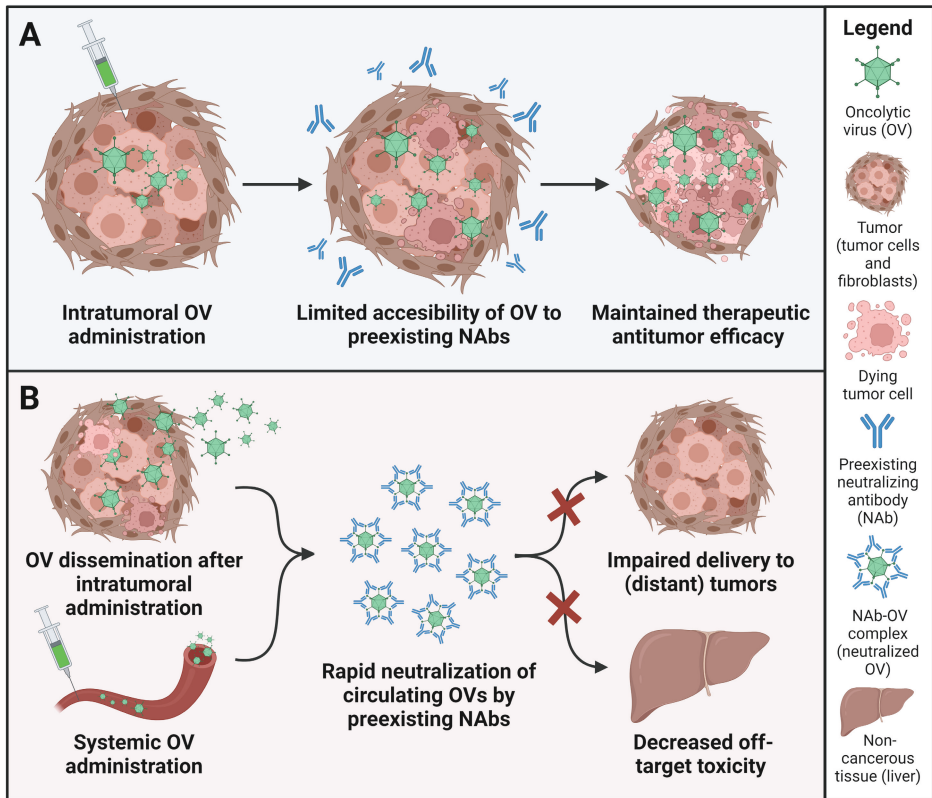
## **THE EFFECT OF PREEXISTING IMMUNITY ON OV INFECTION AND SPREAD**

Until recently, interest regarding the effects of preexisting immunity has been largely focused on the early phases of OV therapy, which comprise the initial infection of tumor cells by the OV, its subsequent spread throughout the circulation, and the dissemination to distant tumors and tissues. Although neutralizing antibodies for commonly used OVs are present in the human population as well as cancer patients (**Figure 1**), their effect on the therapeutic efficacy of OVs is highly dependent on many variables, including the route of administration and the specific OV platform used.

### ***Intratumoral OV therapy is largely unaffected by preexisting immunity***

In the field of OV therapy, local versus systemic delivery is a huge topic of debate (56). Local, intratumoral delivery of OVs is in clinical practice for T-VEC (57,58) and is often used in preclinical studies to ensure efficient delivery to the tumor site (59). Theoretically, intratumorally administered OVs might be less accessible to preexisting antibodies than circulating OVs (**Figure 3A**), although this could vary depending on tumor vascularization (60). Direct cell-to-cell spread after infection with several OVs, including HSV-1, Vaccinia virus, and Measles virus, was shown to be unaffected by the presence of neutralizing antibodies in both *in vitro* and *in vivo* contexts (61,62). However, several other studies have reported that preexisting immunity can limit intratumoral viral replication or spread (63-65). For example, induction of preexisting immunity by intramuscular exposure to

Ad5 before intratumoral injection of this OV into subcutaneous HPD-1NR pancreatic carcinomas, resulted in rapid clearance of viral load from the tumor in hamsters (66).



**Figure 3. Route of administration contributes to the effect of preexisting neutralizing antibodies (NABs) on OV efficacy. (A)** Intratumorally injected OVs might be less accessible to preexisting NABs (in blue), leading to maintained therapeutic antitumor efficacy. **(B)** OVs that disseminate into the circulation after intratumoral injection, as well as OVs that are systemically administered, are susceptible to rapid neutralization. This can limit the delivery efficiency to (distant) tumors, but also decrease off-target toxicity.

Interestingly, although preexisting immunity against Ad5 and other OVs including HSV-1 and Coxsackievirus A21 (CV-A21) can result in reduced intratumoral viral replication, preexisting immunity against these OVs does not mitigate the OV-induced effect on primary tumor growth or animal survival upon intratumoral OV therapy (63-69). These results highlight the discrepancy between viral replication and therapeutic efficacy in the setting of intratumoral administration (70). Indeed, clinical trials investigating the efficacy of various OVs with high seroprevalence that were injected directly into a variety of readily accessible tumors, such as melanomas, have been relatively successful (4,55,71-73). One of these trials, investigating the efficacy of intratumoral Ad5 treatment of pancreatic ductal adenocarcinoma, showed there was no significant correlation between preexisting anti-Ad5 antibody titers and changes in tumor size upon therapy (74). Thus, both the preclinical and clinical data suggest that, even though OV replication

might be decreased, preexisting immunity should generally not be considered an obstacle to primary tumor clearance in the setting of intratumoral OV therapy.

### ***Rapid neutralization of OVs shed by infected tumors prevents viral dissemination***

Although intratumoral OV therapy consists of direct injection of OVs into the tumor microenvironment, spillover and viral shedding as a consequence of oncolysis will introduce OVs into the circulation. These circulating OVs are readily accessible to preexisting antibodies and thus subject to neutralization, which can impact OV therapy in a variety of ways (**Figure 3B**). For instance, in the case of multiple tumors, preexisting immunity might prevent intratumorally administered OVs from disseminating to distant tumors. An example of this was shown to occur upon injection of CV-A21 into one of two bilateral subcutaneous YUMM 2.1 melanomas in immunocompetent C57BL/6 mice (69). For naive animals, viral genomic material was present in the blood and both tumors, but intraperitoneal preexposure to CV-A21 completely precluded viral recovery from the circulation and the distant tumor. Likewise, another study showed that passive immunization with Vaccinia-specific immunoglobulins strongly reduced dissemination to lung, bone, and lymph node metastases in BALB/c mice upon injection of a primary 4T1 mammary carcinoma with luciferase-expressing Vaccinia virus (75). As such, preexisting immunity is likely detrimental to therapeutic efficacy in a setting of metastatic disease, where therapy should affect both the injected and distant tumors.

Importantly, preexisting NAbs can reduce toxicity associated with intratumoral OV therapy by limiting viral dissemination to off-target tissues. This was investigated in a study using an intratumoral injection of subcutaneous PymT-induced breast adenocarcinoma with a luciferase-expressing replication-deficient Ad5 in FVB/n mice (63). Here, intranasal exposure to Ad5 before OV therapy strongly reduced luciferase activity in the liver, which is a major site of Adenovirus off-target toxicity, while only slightly reducing transgene expression in the tumor. Similar results were obtained for intratumoral treatment of subcutaneous HaK kidney tumors with an Ad5 OV in Syrian hamsters (64). Here, intramuscular preexposure to Ad5 completely abrogated recovery of viral genome copies from the liver and lungs, as well as infectious virus from the liver, whereas naive animals exhibited dissemination to these tissues and active viral replication in the liver. Importantly, tumor growth was similarly inhibited for both naive and preexposed animals. Thus, it appears that NAbs prevent OV dissemination to distant tumors upon intratumoral therapy, but can also be beneficial by limiting dissemination and infection of off-target tissues.

### ***The efficacy of systemic OV therapy is abrogated by preexisting immunity***

Clinically speaking, systemic OV administration is often preferable to intratumoral injection, as it limits patient discomfort and allows for the treatment of tumors that are not easily accessible (76). However, since therapeutic efficacy in this context is completely dependent on dissemination via the circulation, preexisting immunity

represents a major hurdle to this route of administration (**Figure 3B**). Indeed, the preclinical efficacy of most systemically-administered OV therapies, including Measles virus, VSV, HSV-1, and Ad5, is severely abrogated by preexisting immunity (77-80). For example, a study investigating the efficacy of intravenous VSV-GFP treatment in BALB/c mice bearing subcutaneous CT26 colon carcinomas demonstrated that intravenous VSV exposure before OV therapy completely abrogated transgene expression and recovery of infectious virus from the tumor, which was not observed in naive animals (81). Similar attenuation was observed upon passive immunization with antibody-containing serum, but not for animals receiving a transfer of T cells from donor mice exposed to VSV. Passive immunization with purified Ad5-specific antibodies was also shown to inhibit intratumoral Ad5 replication and clearance of subcutaneous LNCaP prostate cancer tumors in BALB/c nude mice treated intravenously with Ad5, while Ad5 treatment demonstrated antitumor activity in a setting without Ad5 NAbs (80). As such, the accessibility of these systemically administered OVs to NAbs appears to be the main reason for their diminished therapeutic efficacy in an immunized host.

The specific site of intravenous delivery might be an important consideration for therapeutic outcome, as it influences the effect of preexisting immunity on OV efficacy. This was shown for HSV-1 therapy in BALB/c mice carrying hepatic metastases established by subcapsular injection of CT26 colon carcinoma cells (82). Here, intraperitoneal preexposure attenuated HSV-1-induced tumor clearance upon tail vein, but not portal vein delivery of HSV-1. As delivery into the portal vein reduces the distance to its target, it likely minimizes the window in which preexisting antibodies can abrogate therapeutic efficacy through the neutralization of OVs. Thus, this observation supports a model in which the required distance of OV dissemination is inversely related to the attenuating effect of preexisting immunity. Together, these studies support the role of preexisting antibodies as a likely contributing factor to the limited efficacy of clinical trials investigating systemic OV delivery and show that nuanced consideration of delivery sites is warranted.

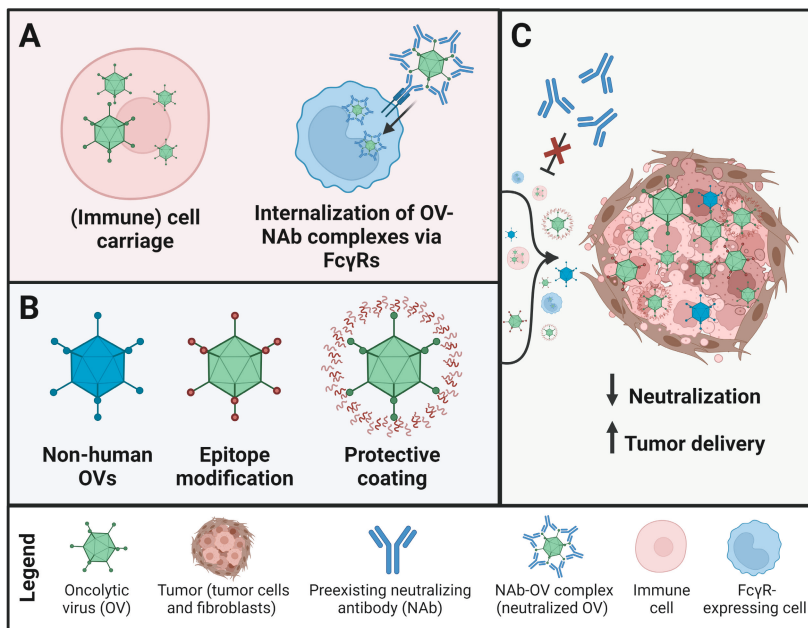
## **EVADING PREEXISTING IMMUNITY FOR IMPROVED OV INFECTION AND SPREAD**

To improve the infection and spread by OVs, many studies have explored modifications of OV therapy to evade neutralization by NAbs (54). Especially in the context of systemic therapy, such strategies might strongly increase therapeutic efficacy.

### ***Cell carriage can rescue the efficacy of systemic OV therapy despite preexisting immunity***

Avoiding recognition of OVs by neutralizing antibodies might be achieved by utilizing infected cells as 'Trojan horses' to deliver OVs to tumors (**Figure 4A, C**). Early clinical trials demonstrated that systemically delivered Reovirus was able to reach and actively

infect distant tumors, despite the presence of Reovirus-specific NABs (83). Interestingly, replication-competent Reovirus could be recovered from circulating PBMCs, granulocytes, and platelets but not plasma. This suggests that immune cell carriage can be employed for shuttling and handing off OV to distant tumors, as a means to evade OV clearance by neutralizing antibodies. Indeed, mechanistic studies have shown that Reovirus can be internalized by various immune cells, including DCs and T cells (84,85). One study assessed the consequences of cell carriage by subcutaneously implanting B16 melanomas, treating C57BL/6 mice intravenously with either free or cell-carried Reovirus, and then assessing the number of metastatic colonies in the tumor-draining lymph node (86). For both naive and Reovirus preexposed animals, Reovirus-loaded mature DCs and T cells outperformed free OVs in limiting lymph node metastases, likely as a result of more efficient draining and thus viral delivery to lymph nodes by immune cells. Similarly, T cells loaded with Measles virus facilitated delivery of Measles virus to tumors in the presence of NABs (87). Other studies have investigated stem cells as potential OV carriers, as they are naturally resistant to chemotherapeutic drugs and can survive in the tumor microenvironment (88). As an example of such a strategy, Ad5-infected neural stem cells were less susceptible to *in vitro* serum neutralization and led to more efficient *in vivo* infection of intracranial GL261 gliomas when delivered in multiple cycles, compared to naked OVs (89).



**Figure 4. Strategies to evade neutralization by preexisting neutralizing antibodies (NABs).** (A) OVs can be carried by various (immune) cells, such as dendritic cells (DCs), T cells or stem cells to avoid neutralization. Alternatively, OV-NAB complexes can be internalized by cells expressing antibody-binding Fc-gamma receptors. (B) Usage of non-human OVs, epitope modification or a protective coating to decrease recognition and clearance by NABs. (C) Employment of evasion strategies described in (A) and (B) lead to decreased neutralization and improved delivery of the OV to the tumor.

Interestingly, when using a cell carrier system for OV delivery, the presence of NAbs might even be beneficial. For instance, antibody-Reovirus complexes can effectively be internalized by human monocytes and delivered to tumor cells, resulting in infection and lysis of Mel-624 melanoma cells. This internalization is mediated via the antibody-binding Fc-gamma receptors (FcγRs), expressed on the surface of monocytes and other immune cells (90). Similarly, antibody-neutralized CV-A21 was shown to be ineffective at killing Mel-624 cells *in vitro* unless carried and handed off by monocytes (90). Furthermore, A549 lung carcinoma cell lines artificially expressing FcγRs have been shown to internalize antibody-neutralized Ad5 (91). The antibody-dependent enhancement (ADE) of viral infection through internalization of antibody-virus complexes by FcγR-expressing cells has been described to occur for a range of viruses, such as Influenza virus, Measles virus, Coronaviruses, and most notably Flaviviruses (92,93). In contrast to Reovirus, these viruses can efficiently replicate in their carrier cells, ultimately resulting in cell death. Since this broadens viral tropism and eliminates immune cells, ADE is often associated with poorer disease outcomes. As such, the ability of some OVs to productively replicate in FcγR-expressing cells might preclude them from beneficial cell carriage, as it would result in the rapid elimination of carrier cells before they can facilitate viral dissemination to distant tumors. Nevertheless, it appears that delivery of OVs via (immune) cell carriage could be a promising new approach for systemic delivery of OVs, especially in preexposed individuals.

### ***Non-human OVs demonstrate oncolytic activity towards human tumors but are less susceptible to neutralization***

Another way to avoid recognition by preexisting immune responses is the use of alternative viral strains, which are sufficiently different from their human-infecting homologs but also display oncolytic effects (**Figure 4B, C**). The capacity of non-human OVs to kill human tumor cells has been demonstrated for various viruses, such as an HSV-1 virus derived from goats that was able to replicate in different human cell lines and induce apoptosis (94,95). Additionally, Adenoviruses isolated from non-human primates were shown to effectively infect and kill a wide range of human cancer cell lines *in vitro*, while not being neutralized by pooled human donor serum (96). Similarly, an avian Reovirus was able to infect hepatocellular carcinoma cells and induce apoptosis *in vitro* but is likely less susceptible to neutralization in humans, since structural analysis demonstrated that its neutralizing epitopes were distinctly different from its human homolog (97). Other examples of non-human virus species that are in development as oncolytic agents have been described elsewhere (98,99). The (pre)clinical efficacy of most of these non-human viruses remains to be proven, but they represent an attractive alternative to currently used OVs.

### ***Genetic modification limits neutralization by OV-specific preexisting antibodies***

Alternatively, antibody-binding sites of OVs can be altered by genetic modification, preventing neutralization by preexisting antibodies (**Figure 4B, C**). For example, the introduction of point mutations in the gD glycoprotein of HSV-1 was shown to result

in increased resistance to *in vitro* neutralization by monoclonal antibodies (100). More radical modification is also possible by exchanging surface glycoproteins of OV with those from other viruses with lower rates of preexposure in the population. This so-called envelope exchange has been utilized for the generation of chimeric Measles virus strains with surface proteins originating from the Canine Distemper virus, which retain their oncolytic activity *in vitro* and *in vivo* (77,79,101). Indeed, this modified Measles virus demonstrated potent oncolytic antitumor efficacy in athymic nude mice bearing intraperitoneal SKOV3.ip1 ovarian cancers and passively immunized with measles-immune human antibody serum, while the efficacy of the non-modified Measles virus was strongly diminished (79). Similar chimerism has also been explored for Ad5, by switching its serotype to that of the related Ad3 or Ad35 to evade neutralization (102-104).

### ***Shielding or coating of OVs prevents immune recognition***

Modification of neutralizing epitopes on OVs or the use of OVs from other hosts thus appear promising for the evasion of preexisting immunity present in the population. Nevertheless, both modified and non-human OVs will likely still be affected by the antiviral immune response induced by repeated therapeutic administrations. Thus, shielding surface epitopes of OVs with a non-immunogenic coat to prevent recognition might be an alternative strategy (**Figure 4B, C**). This can be achieved by genetic modification of the OV, as was shown for the insertion of an albumin-binding domain in the main capsid protein of Ad5 (105). Intravenous administration of a luciferase-expressing Ad5 virus into nude mice bearing subcutaneous B16-CAR melanomas that were intraperitoneally preexposed to Ad5 led to complete neutralization, as the Ad5-mediated luciferase expression within tumors was completely abolished. In contrast, the albumin-binding Ad5 did not suffer from significant loss of luciferase signal in tumors. Similarly, in nude mice bearing subcutaneous A549 or Sk-mel28 tumors that were intraperitoneally preexposed to Ad5, the oncolytic antitumor efficacy of intravenously administered albumin-binding Ad5 was maintained while the Ad5 without the albumin-binding domain was completely inefficacious. As another example, Vaccinia virus has been successfully modified to increase the release of so-called extracellular enveloped viruses (EEVs) upon infection, which have an additional membrane layer and are thereby less susceptible to immune-mediated clearance compared to Vaccinia virus particles themselves (75). This EEV-enhanced Vaccinia virus displayed improved spread to metastases in the lungs and lymph nodes after intratumoral delivery in BALB/c mice inoculated with 4T1 tumors in the mammary fat pad, compared to a Vaccinia virus variant that was less capable to produce EEVs. Similarly, a significant survival advantage was provided by the EEV-enhanced strain over the wild-type virus in BALB/c mice bearing subcutaneous JC tumors.

Alternatively, OVs can be artificially coated by the attachment of ionic polymers, graphene sheets, or liposomes to shield them from antibody recognition (78,106). For example, multilayer ionic polymer coating of Measles virus resulted in improved

control of subcutaneous LL/2-CD64 lung cancer tumors compared to the non-coated virus in Measles-preimmunized C57BL/6N mice (107). In another study, shielding of Ad11 using a hybrid membrane comprised of artificial lipid membranes and red blood cell membranes protected the virus from neutralizing antibodies, prolonged its circulation, and enhanced its antitumor efficacy in the murine TC1 lung cancer model (108). Further (pre)clinical evaluation of the strategies described above would be interesting to optimally enhance the efficacy of (systemically delivered) OV therapy in preexposed patients.

## **EFFECTS OF PREEXISTING IMMUNITY ON THE OV THERAPY-INDUCED IMMUNE RESPONSE**

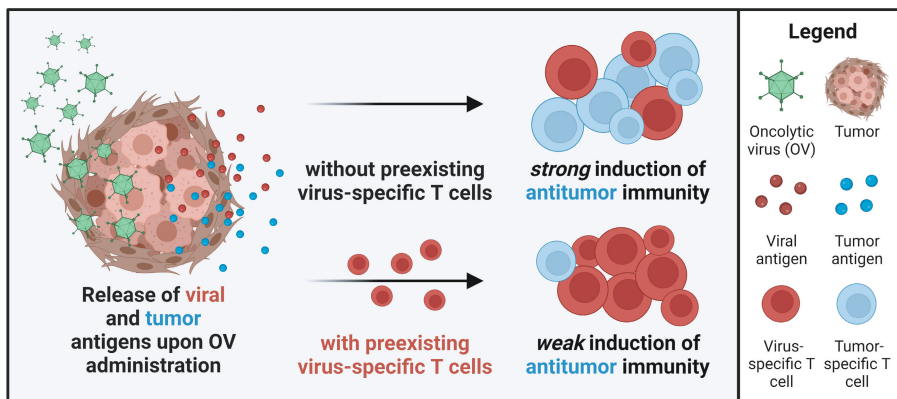
Besides viral replication and oncolysis, the induction of a potent immune response is a second, but equally important pillar of OV therapy (45) (see also section 1). However, if and how the presence of preexisting immunity also affects the OV-induced immune response remains underexplored. Here, we gathered (pre)clinical evidence that describes the effect of preexisting immunity regarding the induction of virus- and tumor-specific immune responses.

### ***Repeated OV exposure can limit the induction of a tumor-specific immune response***

Indications that preexisting immunity can affect OV-induced immune responses can be derived from studies utilizing multiple dosages of OVs. Specifically, it has been shown that homologous boosting regimens impair the induction of a tumor-specific T-cell response, in contrast to heterologous prime-boost schedules utilizing a combination of distinct OV platforms. An example of this was shown for intratumoral OV therapy of hamsters with subcutaneously implanted HaK kidney tumors or HPD-1NR pancreatic carcinomas (109). In both models, a heterologous treatment schedule comprising three intratumoral Ad5 injections followed by three intratumoral Vaccinia injections displayed significantly superior antitumor efficacy compared to 6 doses of either virus alone. This heterologous OV therapy resulted in improved induction of tumor-specific T cells compared to treatment with either virus alone, and these T cells were responsible for therapeutic efficacy since the depletion of CD3<sup>+</sup> T cells completely abrogated the antitumor effect of this combination therapy. OVs encoding a transgene appear to be similarly affected by dosage regimens. For instance, in a CT26 metastasis model where tumors express  $\beta$ -galactosidase, two intravenous doses of either  $\beta$ -galactosidase-expressing Vaccinia virus or the related  $\beta$ -galactosidase-expressing Fowlpox virus resulted in inferior overall survival compared to sequential treatment with both viruses (110). Heterologous boosting led to higher  $\beta$ -galactosidase-specific CD8<sup>+</sup> T-cell responses compared to homologous boosting, and homologous boosting was associated with the induction of a strong antiviral antibody response.



Although repeated OV administration can hamper the OV-induced tumor-specific T-cell response, evidence for the mechanisms underlying this problem remains elusive. This phenomenon could simply be explained by lower clearance of the OV by NABs, but another possible explanation could be derived from the immunodominance of previously encountered viral T-cell epitopes. Besides the notion that most viral epitopes are inherently more immunogenic than most tumor epitopes, an OV-specific T-cell response is boosted upon the reintroduction of previously recognized viral epitopes. Both aspects might result in an immunodominant OV-specific T-cell response over the tumor-specific T-cell response (**Figure 5**). This phenomenon, sometimes referred to as ‘original antigenic sin’ (111), has been extensively studied for vector-based vaccines and Influenza infections but has not gained a lot of attention in the field of OV research (112). Nevertheless, as similar viral strains are often used in both fields, data showing problematic viral epitope immunodominance for vector vaccines highlights current gaps in OV research and might indicate shared mechanisms. Of note, it could be that different OV platforms vary in their inherent immunogenicity, making them more or less dominant over the tumor-specific immune response. Indeed, research on viral vectors has shown that viral backbones can differ in the type and potency of immune responses they induce (113), indicating the same might be true for OVs.



**Figure 5. Immunodominance of OV-specific T-cell responses over tumor-specific T-cell responses.** Viral epitopes are often more immunogenic compared to most tumor epitopes. Additionally, in the setting of preexisting immunity or repeated dosage, the preexisting OV-specific T-cell response is boosted upon repeated recognition of the viral epitopes. These combined aspects may result in an impaired induction of an antitumor T-cell response compared to a strong virus-specific T-cell response.

The possible immunodominance of the viral backbone over transgenes could especially be relevant for OVs which encode tumor antigens. For instance, investigation of intramuscular delivery of Hepatitis B surface antigen (HBsAg) and ovalbumin (OVA) antigen in BALB/c mice using an Ad5-based vector revealed prior exposure to Ad5 strongly reduced the HBsAg- and OVA-specific CD8<sup>+</sup> T-cell responses (114). Instead, isolated CD8<sup>+</sup> T cells were mainly reactive to Ad5 epitopes. Skewing of immunity towards an antiviral response was replicated in antibody-deficient IgH<sup>-/-</sup> mice, indicating it is the

established Ad5-specific cellular immunity, and not the Ad5-specific humoral response, that limits the priming and expansion of HBsAg/OVA-specific CD8<sup>+</sup> T cells. Similarly, induction of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses specific for an Influenza virus antigen, which was intramuscularly delivered using a Vaccinia virus vector, was completely abrogated by prior exposure to Vaccinia virus (115). Highlighting the relevance of such preclinical observations, clinical data suggests similar immunodominance occurs in humans. For example, several trials of Ad-vectored vaccines have reported correlations between preexisting Ad-specific CD4<sup>+</sup> T cells (116,117) or antibodies (118) and strongly decreased induction of CD4<sup>+</sup> T-cell, CD8<sup>+</sup> T-cell and antibody responses directed against the delivered vaccine antigen. Although these studies utilized Adenovirus to deliver Ebolavirus and HIV epitopes irrelevant to OV therapy, these observations might be relevant to the field of OV research. Furthermore, the discussed data on OV boosting regimens might suggest that preexisting immunity could also affect responses to tumor antigens released after oncolysis, due to the simultaneous release of viral epitopes.

Intramuscular, intravenous, and intratumoral OV administration are likely to result in distinct dynamics of viral epitope exposure to the immune system and thus influence the development of antiviral immunodominance, but a direct comparison of routes of administration has yet to be performed. Regardless of the underlying mechanisms, heterologous prime-boost regimens appear to be beneficial for some OV therapies by improving tumor-specific immune responses and tumor clearance. Consequently, clinical trials of such strategies are promising and currently ongoing. For example, sequential systemic therapy with Ad5 and Maraba virus, both encoding the tumor antigen MAGE-A3, showed preclinical efficacy and is currently being tested for the treatment of advanced metastatic solid tumors and non-small cell lung cancer (NCT02285816, NCT02879760) (119-121).

***Preexisting OV-specific immunity can also improve therapeutic anticancer efficacy by enhancing tumor-specific T-cell responses***

The data described above suggested that OV administration might result in the dominance of OV-specific T-cell responses over tumor-specific T-cell responses upon repeated exposure. However, other studies suggest that preexisting OV-specific immunity does not hamper, but can actually promote the induction of a systemic tumor-specific immune response. An example of this was shown for immunocompetent C57BL/6J mice with subcutaneously implanted bilateral B16.F10 melanomas, of which one was injected with NDV (122). In this setting, prior subcutaneous footpad exposure to NDV led to improved control of tumor size as well as extended survival upon intratumoral NDV treatment, even though viral replication was compromised. For both the injected and distant tumor, the ratio of conventional CD4<sup>+</sup> T cells over regulatory T cells as well as the expression of genes related to immune-mediated cytotoxicity were strongly increased by preexposure to NDV. In the distant, but not the injected tumor of preexposed animals, an increase in CD8<sup>+</sup> T-cell influx could be observed, which was not the case for the distant tumors of naive animals. Prior NDV exposure did not significantly increase

the amount of virus-specific CD8<sup>+</sup> T cells in the spleen but instead caused a strong increase in the amount of tumor-specific CD8<sup>+</sup> T cells. CD8<sup>+</sup> T-cell depletion completely abrogated the antitumor effect of NDV in immunized mice, suggesting that CD8<sup>+</sup> T cells were indispensable for the therapeutic efficacy of NDV in a preexposed setting. Similar effects of preexisting immunity on therapeutic OV efficacy were recently shown for the intratumoral treatment of BALB/c mice with subcutaneously implanted bilateral CT26 colon carcinomas using a highly modified HSV-1, expressing several cytokines and a PD-L1 blocking peptide (123). Control of both the injected and distant tumors was improved by subcutaneous preexposure to HSV-1, as was overall survival. Strikingly, the outgrowth of the distant tumor was completely unaffected by intratumoral OV therapy of the local tumor in naive animals, showing preexisting immunity was required for systemic efficacy in this setting. Gene expression profiling of tumors again revealed a skewing toward cytotoxic and inflammatory responses. Additionally, isolated splenocytes from preexposed mice were more reactive to tumor cells compared to splenocytes from naive animals, indicating an increased induction of tumor-specific immunity.

Thus, it appears that preexisting immunity can also promote the induction of a tumor-specific immune response upon therapy with these OVs. These tumor-specific responses have a systemic impact with efficacy on distant tumors and could thus have the potential to treat metastatic disease. Whether this phenomenon extends to other OV platforms and its underlying mechanisms, however, remains to be explored. One possibility could be that preexisting antiviral CD4<sup>+</sup> T cells aid the development of tumor-specific CD8<sup>+</sup> T cell responses. CD4<sup>+</sup> T-cell help has been well established as a crucial factor in the induction of robust CD8<sup>+</sup> T-cell responses but is generally considered to be restricted to responses specific to the same antigen (124). Nevertheless, some studies have indicated that CD4<sup>+</sup> T cells might also mediate more general immune-stimulating effects upon activation by their cognate antigen, such as an increase in naive lymphocyte recruitment to lymph nodes (125). Indeed, it was recently shown in C57BL/6 mice that were intramuscularly vaccinated with tetanus toxoid before intratumoral OVA-coated Ad5 therapy, that additional coating of the Ad5 with major histocompatibility complex (MHC) class II-restricted tetanus toxoid peptides led to increased infiltration of tumor-specific CD8<sup>+</sup> T cells into subcutaneous B16.OVA melanomas (126). As the tetanus toxoid coating resulted in potent stimulation of preexisting pathogen-specific CD4<sup>+</sup> T-helper cells, it appears likely that pathogen-specific CD4<sup>+</sup> T-cell help can potentiate tumor-specific CD8<sup>+</sup> T-cell responses. In another study, it was revealed that prior vaccination against poliovirus substantially improved the antitumor efficacy of intratumoral polio treatment in C57BL/6 mice bearing murine melanoma B16.F10 tumors, and that this antitumor effect was mediated by the recall of CD4<sup>+</sup> T cells and the induction of tumor-specific T cells that could delay tumor outgrowth in naive mice after adoptive cell transfer (127). So far, preexisting virus-specific CD4<sup>+</sup> T cells have been largely overlooked in the OV research field, but these observations suggest that they might play an important part in modulating the OV-induced immune response, especially in a setting where preexposure has occurred.

## EXPLOITING PREEXISTING VIRUS-SPECIFIC IMMUNITY FOR EFFECTIVE ANTICANCER IMMUNOTHERAPY

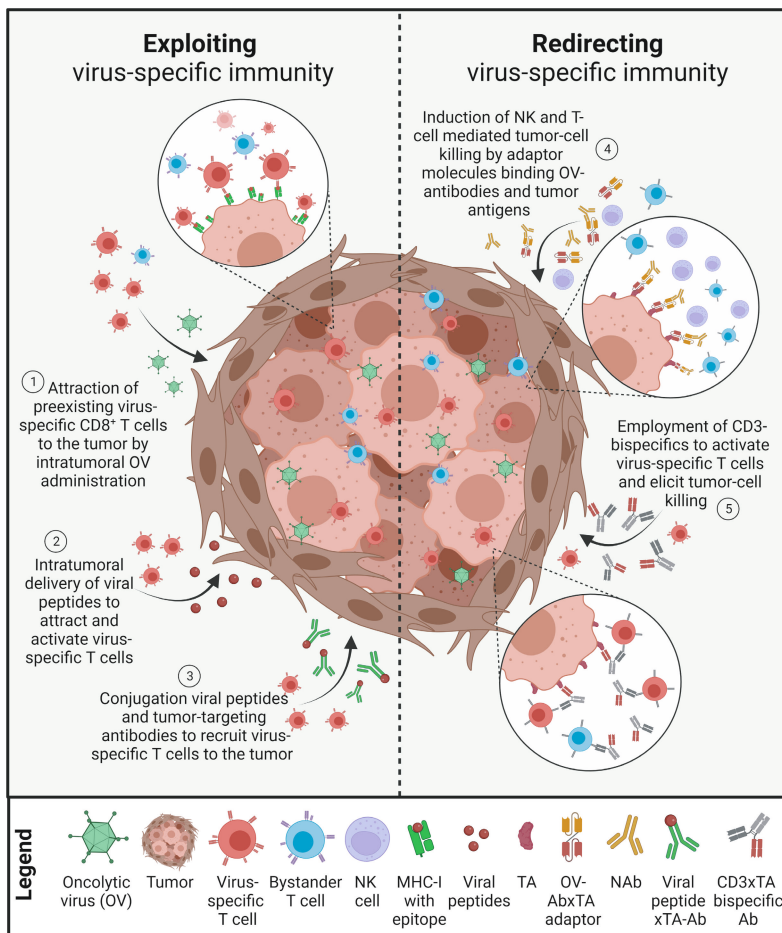
Regardless of the induction of a tumor-specific immune response in a preexposed setting, increasing amounts of evidence suggest that preexisting antiviral effector responses might also be engaged to directly contribute to tumor clearance and thus therapeutic efficacy. Studies have shown that antiviral CD8<sup>+</sup> T cells commonly survey a range of both murine and human tumors, including melanomas, brain metastases, endometrial, lung, and colorectal cancers (128,129). Upon immune cell profiling, tumor-specific CD8<sup>+</sup> T cells found in patient tumors expressed high levels of T-cell exhaustion, likely as a result of chronic antigen exposure in the tumor (129). CD8<sup>+</sup> T cells specific for common viral pathogens, such as Cytomegalovirus (CMV), Epstein-Barr virus (EBV), or Influenza virus, on the other hand, exhibited phenotypes more in line with active effector cells. Indeed, virus-specific T cells, as determined by staining with HLA tetramers specific for these viruses, could be potently activated after isolation from tumor tissue by providing relevant viral peptides (128). Various strategies are described to employ antiviral T cells for anticancer therapy, either by reactivation using their cognate antigens, or in a specificity-independent manner.

### ***Preexisting antiviral T cells can be activated and engaged for anticancer therapy***

The engagement of preexisting antiviral T cells for antitumor activity is an appealing avenue for immunotherapy, in particular for the treatment of low-immunogenic tumors (**Figure 6**). One way to achieve this is by delivering viral epitopes into the tumor, resulting in the activation of antiviral T cells present in the tumor microenvironment. For example, preexisting Reovirus-specific CD8<sup>+</sup> T cells, induced by vaccination with a synthetic viral peptide containing the Reovirus CD8<sup>+</sup> T-cell epitope, were efficiently recruited into subcutaneous KPC3 pancreatic tumors upon intratumoral injection of Reovirus (53). In this study, the presence of this preinstalled pool of Reovirus-specific effector cells significantly delayed tumor outgrowth after intratumoral Reovirus administration, an effect not observed when Reovirus was administered to naive animals. Similar effects on tumor growth were observed in animals that were immunized with Reovirus before vaccination, showing that vaccine-mediated boosting of preexisting Reovirus-specific CD8<sup>+</sup> T cells can improve OV therapeutic efficacy. Similarly, intratumoral delivery of the Vaccinia virus-derived B8R protein by a recombinant adeno-associated virus reactivated preinduced Vaccinia-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells and retarded outgrowth of murine DT6606 pancreatic tumors (130).

Besides direct infection with a virus, other innovative strategies can also be employed to reactivate virus-specific T cells. Although these studies often investigate the use of non-OV-specific T cells, these observations should also be instructive for the employment of preexisting OV-specific T cells. For example, injection of B16 melanomas with the viral peptide SIINFEKL resulted in improved tumor control and survival over an irrelevant peptide in C57BL/6j mice that had previously received a transfer of OT-1 CD8<sup>+</sup> T cells

which target this epitope (128). Similarly, intratumoral injection of murine CMV (MCMV)-derived T-cell epitopes triggered the expansion of MCMV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells in TC1-bearing immunocompetent mice that were preexposed to MCMV (131). Injection of MHC-I-restricted MCMV epitopes into TC1 tumors induced a T-cell/IFN- $\gamma$  signature, delayed tumor outgrowth, and improved survival. Expanding on such an approach is the idea that the conjugation of virus-derived epitopes to tumor-targeting antibodies might improve their specificity and facilitate systemic efficacy. This was demonstrated in a study where CMV-derived epitopes conjugated to an antibody targeting the tumor antigen MMP14 could be used for efficient recruitment of preexisting antiviral CD8<sup>+</sup> T cells towards various MMP14-expressing tumors (132). This resulted in improved control of orthotopic MDA-MB-231 breast tumors, as well as orthotopic SNU-475 liver or subcutaneous MGH-1 lung tumors.



**Figure 6. Strategies to exploit or redirect (preexisting) virus-specific T cells for anti-tumor immunotherapy.** Multiple avenues can be employed to exploit the specificity of (oncolytic) virus-specific immunity for anticancer immunotherapy. (1) Preexisting OV-specific T cells can be attracted to the tumor by intratumoral OV administration and activated by presentation of OV >>

- >> epitopes on the surface of tumor cells in MHC-I proteins. (2) Intratumoral delivery of viral peptides leads to activation of intratumoral virus-specific T cells. (3) Complexes of viral peptides together with a tumor-targeting antibody can recruit OV-specific T cells to the tumor. (4) Adaptor molecules binding to both OV-specific antibodies and tumor antigens induce NK- and T-cell-mediated killing of tumor cells. (5) Utilization of CD3-bispecific antibodies transforms OV-specific T cells into tumor-attacking T cells. NK; natural killer, MHC-I; major histocompatibility molecule class I; TA; tumor antigen, Ab; antibody.

A similar principle was applied in a model where immunodeficient female non-obese diabetic (NOD).Cg-Prkdc<sup>scid</sup>Il2rg<sup>tm1Sug</sup>/Shijic (NOG) mice bearing MDA-MB-231 breast cancer xenografts received an adoptive transfer of expanded human EBV-specific CD8<sup>+</sup> T cells, which were subsequently directed to the tumor by use of immunoconjugates called Antibody-Targeted Pathogen-derived Peptides (ATPPs) (133). Here, MHC class I peptides are conjugated to antibodies specific for a tumor antigen that is expressed on the tumor cell surface. This tumor-specific delivery of EBV peptides activated EBV-specific T cells and delayed tumor outgrowth in combination with PD-1 checkpoint blockade. Similarly, in NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/Szj (NSG) mice bearing the same MDA-MB-231 tumors, CMV-specific T cells could be redirected to exert antitumor efficacy via a CD8<sup>+</sup> T-cell epitope-delivering antibody (termed TEDbody), which was engineered to deliver a viral MHC-I epitope peptide into the cytosol of target tumor cells by fusion with a tumor-specific cytosol-penetrating antibody (134).

Thus, delivery of viral epitopes into the tumor microenvironment, through a variety of ways, can be utilized to engage preexisting antiviral T cell populations for antitumor effect. An exciting strategy involves the exploitation of ‘molecular mimicry’, where preexisting virus-specific T cells can demonstrate cross-reactivity toward tumors after restimulation with tumor-specific antigens that display high similarities to their cognate viral antigens (135). For instance, in a cohort of melanoma patients with high anti-CMV antibody levels, it was suggested that molecular mimicry between CMV and tumor antigens played a role in the response to anti-PD1 therapy blockade by activation of cross-reactive T cells. Another enticing opportunity is the reactivation of T cells that are induced by exposure to a common virus or established antiviral vaccines, which have already been abundantly tested for clinical safety and are administered to a majority of the human population. As examples of this, T cells specific for Influenza virus (136), Yellow Fever virus (137), or even SARS-CoV-2 (138) might be employed for anticancer therapy.

### ***Redirecting the specificity of preexisting virus-specific responses for their use as anticancer effector cells***

As an alternative approach to using the specificity of preexisting antiviral immune responses, the inherent specificities could also be redirected to the tumor by using bispecific molecules (139). Such retargeting of preexisting virus-specific antibodies and T cells for antitumor activity using bispecific molecules might be used to improve OV efficacy (140) (**Figure 6**). For instance, a recent study described the design of a bispecific adaptor molecule containing an Ad5 antibody-binding epitope and a domain

that binds polysialic acid, a surface adhesion molecule associated with a range of cancers (141). Immunocompetent C57BL/6 mice were immunized with Ad5 to develop anti-Ad5 antibodies, which were subsequently recruited to the tumor with the bispecific adaptor molecule. This treatment led to improved tumor control and survival of mice with subcutaneous polysialic acid-expressing MC38 colon carcinomas, CMT-64 lung carcinomas, and B16F10 melanomas compared to naive mice. Further studies in MC38 tumors established a model in which the retargeted Ad5 antibodies recruited and activated NK cells, which mediated initial tumor cell killing through antibody-dependent cellular cytotoxicity (ADCC) and thereby induced the priming of a tumor-specific CD8<sup>+</sup> T-cell response (142).

Besides virus-specific antibodies, preexisting OV-specific T cells can also be directly recruited for antitumor efficacy using CD3-bispecific molecules (also known as bispecific T-cell engagers (BiTEs)). For instance, intratumoral Reovirus administration to subcutaneous KPC3 pancreatic tumors expressing tumor antigen TRP1 led to a strong influx of virus-specific CD8<sup>+</sup> T cells, which could be subsequently engaged for delayed tumor growth by intraperitoneal administration of a bispecific antibody targeting both CD3 and TRP1 (52). When tested in a bilateral model, this combination therapy led to delayed tumor growth for both the injected and non-injected distant tumors, showing such strategies could be efficacious in a setting of metastatic disease. Current undertakings in this field especially involve the use of OVs encoding BiTEs, where the OV acts both as an immunostimulatory agent, as well as a vector for BiTE delivery into the tumor (143). Together, these results showcase the potential of bypassing the specificity of preexisting antiviral immunity using bispecific molecules for effective anticancer therapy.

## **CONCLUDING REMARKS**

In this review, we discussed how preexisting immunity against OVs can act as a barrier, but also as a bridge to effective anticancer therapy. As is evident from the data described here, a preexisting OV-specific humoral response against commonly-used OVs might limit viral replication and spread, especially when the OV is administered intravenously. Importantly, even for OVs that do not abundantly circulate in the human population, the observations discussed here are highly relevant, as therapeutic regimens usually entail multiple OV administrations. Each dose will invariably lead to the development of an antiviral immune response that modulates the efficacy of the next round of therapy.

Effects of preexisting immunity on OV infection and spread have been relatively well explored and suggest that, although various OV modifications can help evade a preexisting immune response, a nuanced case-by-case assessment appears warranted and variables such as the location of the tumor(s), the specific OV used, as well as

the route of OV administration should be taken into account. For example, treatment of a single, easily accessible tumor by intratumoral OV injection would likely not be compromised by preexisting immunity. In the case of metastatic disease, on the other hand, the therapeutic efficacy of both intratumoral and systemic OV administration will be strongly limited by preexisting immunity, making modifications to evade it beneficial or even necessary. Currently, a variable that remains largely unexplored in this context is the confounding effect of tumor location. As discussed, the distance between the site of intravenous OV administration and the target tumor appears to modulate the effect of preexisting immunity on therapeutic efficacy (82), indicating administration sites should be optimized based on tumor localization.

While, generally speaking, preexisting humoral immunity is considered to be a barrier to effective anticancer OV therapy and should be circumvented, preexisting OV-specific cellular immune responses might rather be considered a beneficial factor for OV therapy. Additionally, the route of OV administration, which has been abundantly explored and discussed here in the context of OV infection and spread, remains strongly underappreciated regarding its effect on the induced immune response. Vaccine studies have uncovered clear evidence showing that the site of administration is a crucial determinant of the type and quality of subsequently induced responses (144), highlighting the need for evaluation of this factor in OV research. Regardless of its effects on the induction of a tumor-specific immune response, exciting novel data suggests preexisting OV-specific adaptive immunity can be engaged for direct antitumor effects. However, careful investigation is warranted, since preexisting OV-specific T cells might also be involved in inducing viral clearance (66). Further research in the field of OV research should elucidate how OV replication, the OV-induced immune response, and the ultimate therapeutic effects of OVs all interrelate, and how both preexisting humoral and adaptive immunity influence these aspects.

In conclusion, consideration of preexisting immunity is crucial in realizing the full potential of the highly promising therapeutic implementation of OVs. Future investigation of the current gaps in knowledge highlighted here should yield a more complete understanding of the topic, ultimately allowing for better and more personalized OV therapies.

## DECLARATIONS

**CRedit author statement.** *Christianne Groeneveldt*: Conceptualization, Literature collection, Writing – original draft preparation, Visualization, Writing – review & editing. *Jasper van den Ende*: Conceptualization, Literature collection, Writing – original draft preparation, Visualization, Writing – review & editing. *Nadine van Montfoort*: Conceptualization, Writing – review & editing, Supervision.



**Funding.** This work was financially supported by a PhD fellowship from Leiden University Medical Center (to C.G.) and the Support Casper campaign by the Dutch foundation 'Overleven met Alveesklierkanker' (supportcasper.nl) (SOAK 22.02, to N.v.M). All figures were created with BioRender software (BioRender.com).

**Declarations of Competing Interest.** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## REFERENCES

1. Yun C-O, Hong J, Yoon A-R. Current clinical landscape of oncolytic viruses as novel cancer immunotherapeutic and recent preclinical advancements. **2022**;13
2. Macedo N, Miller DM, Haq R, Kaufman HL. Clinical landscape of oncolytic virus research in 2020. *Journal for ImmunoTherapy of Cancer* **2020**;8:e001486
3. Kohlhapp FJ, Kaufman HL. Molecular Pathways: Mechanism of Action for Talimogene Laherparepvec, a New Oncolytic Virus Immunotherapy. *Clin Cancer Res* **2016**;22:1048-54
4. Andtbacka RHI, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, *et al.* Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. *J Clin Oncol* **2015**;33:2780-8
5. Ferrucci PF, Pala L, Conforti F, Cocorocchio E. Talimogene Laherparepvec (T-VEC): An Intralesional Cancer Immunotherapy for Advanced Melanoma. *Cancers* **2021**;13:1383-
6. Lauer UM, Beil J. Oncolytic viruses: challenges and considerations in an evolving clinical landscape. *Future Oncology* **2022**;18:2713-32
7. Nwanegbo E, Vardas E, Gao W, Whittle H, Sun H, Rowe D, *et al.* Prevalence of Neutralizing Antibodies to Adenoviral Serotypes 5 and 35 in the Adult Populations of The Gambia, South Africa, and the United States. *Clinical and Vaccine Immunology* **2004**;11:351-7
8. Hemminki O, Parviainen S, Juhila J, Turkki R, Linder N, Lundin J, *et al.* Immunological data from cancer patients treated with Ad5/3-E2F-Δ24-GMCSF suggests utility for tumor immunotherapy. *Oncotarget* **2015**;6:4467-81
9. Fueyo J, Gomez-Manzano C, Alemany R, Lee PSY, McDonnell TJ, Mitlianga P, *et al.* A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. *Oncogene* **2000**;19:2-12
10. Mantwill K, Klein FG, Wang D, Hindupur SV, Ehrenfeld M, Holm PS, *et al.* Concepts in Oncolytic Adenovirus Therapy. *Int J Mol Sci* **2021**;22:10522-
11. Bradley H, Markowitz LE, Gibson T, McQuillan GM. Seroprevalence of Herpes Simplex Virus Types 1 and 2--United States, 1999-2010. *Journal of Infectious Diseases* **2014**;209:325-33
12. Puzanov I, Milhem MM, Minor D, Hamid O, Li A, Chen L, *et al.* Talimogene Laherparepvec in Combination With Ipilimumab in Previously Untreated, Unresectable Stage IIIB-IV Melanoma. *Journal of Clinical Oncology* **2016**;34:2619-26
13. Aldrak N, Alsaab S, Algethami A, Bhere D, Wakimoto H, Shah K, *et al.* Oncolytic Herpes Simplex Virus-Based Therapies for Cancer. *Cells* **2021**;10:1541-
14. Costantino V, Trent MJ, Sullivan JS, Kunasekaran MP, Gray R, MacIntyre R. Serological Immunity to Smallpox in New South Wales, Australia. *Viruses* **2020**;12:554-
15. Downs-Canner S, Guo ZS, Ravindranathan R, Breitbach CJ, O'Malley ME, Jones HL, *et al.* Phase 1 Study of Intravenous Oncolytic Poxvirus (vvDD) in Patients With Advanced Solid Cancers. *Molecular Therapy* **2016**;24:1492-501
16. Hwang T-H, Moon A, Burke J, Ribas A, Stephenson J, Breitbach CJ, *et al.* A Mechanistic Proof-of-concept Clinical Trial With JX-594, a Targeted Multi-mechanistic Oncolytic Poxvirus, in Patients With Metastatic Melanoma. *Molecular Therapy* **2011**;19:1913-22
17. Guo ZS, Lu B, Guo Z, Giehl E, Feist M, Dai E, *et al.* Vaccinia virus-mediated cancer immunotherapy: cancer vaccines and oncolytics. *J Immunother Cancer* **2019**;7:6
18. Lebo EJ, Kruszon-Moran DM, Marin M, Bellini WJ, Schmid S, Bialek SR, *et al.* Seroprevalence of Measles, Mumps, Rubella and Varicella Antibodies in the United States Population, 2009–2010. *Open Forum Infectious Diseases* **2015**;2:ofv006-ofv
19. Galanis E, Hartmann LC, Cliby WA, Long HJ, Peethambaram PP, Barrette BA, *et al.* Phase I Trial of Intraperitoneal Administration of an Oncolytic Measles Virus Strain Engineered to Express Carcinoembryonic Antigen for Recurrent Ovarian Cancer. *Cancer Research* **2010**;70:875-82
20. Lin L-T, Richardson C. The Host Cell Receptors for Measles Virus and Their Interaction with the Viral Hemagglutinin (H) Protein. *Viruses* **2016**;8:250-

21. Allagui F, Achard C, Panterne C, Combredet C, Labarrière N, Dréno B, *et al.* Modulation of the Type I Interferon Response Defines the Sensitivity of Human Melanoma Cells to Oncolytic Measles Virus. *Current Gene Therapy* **2017**;16:419-28
22. Engeland CE, Ungerechts G. Measles Virus as an Oncolytic Immunotherapy. *Cancers* **2021**;13:544-
23. Tai JH, Williams JV, Edwards KM, Wright PF, Crowe JJE, Dermody TS. Prevalence of Reovirus-Specific Antibodies in Young Children in Nashville, Tennessee. *The Journal of Infectious Diseases* **2005**;191:1221-4
24. White CL, Twigger KR, Vidal L, De Bono JS, Coffey M, Heinemann L, *et al.* Characterization of the adaptive and innate immune response to intravenous oncolytic reovirus (Dearing type 3) during a phase I clinical trial. *Gene Therapy* **2008**;15:911-20
25. Lolkema MP, Arkenau H-T, Harrington K, Roxburgh P, Morrison R, Roulstone V, *et al.* A Phase I Study of the Combination of Intravenous Reovirus Type 3 Dearing and Gemcitabine in Patients with Advanced Cancer. *Clinical Cancer Research* **2011**;17:581-8
26. Vidal L, Pandha HS, Yap TA, White CL, Twigger K, Vile RG, *et al.* A Phase I Study of Intravenous Oncolytic Reovirus Type 3 Dearing in Patients with Advanced Cancer. *Clinical Cancer Research* **2008**;14:7127-37
27. Comins C, Spicer J, Protheroe A, Roulstone V, Twigger K, White CM, *et al.* REO-10: A Phase I Study of Intravenous Reovirus and Docetaxel in Patients with Advanced Cancer. *Clinical Cancer Research* **2010**;16:5564-72
28. Shmulevitz M, Pan L-Z, Garant K, Pan D, Lee PWK. Oncogenic Ras Promotes Reovirus Spread by Suppressing IFN- $\beta$  Production through Negative Regulation of RIG-I Signaling. *Cancer Res* **2010**;70:4912-21
29. Müller L, Berkeley R, Barr T, Ilett E, Errington-Mais F. Past, Present and Future of Oncolytic Reovirus. *Cancers* **2020**;12:3219
30. Cline BL. Ecological Associations of Vesicular Stomatitis Virus in Rural Central America and Panama. *The American Journal of Tropical Medicine and Hygiene* **1976**;25:875-83
31. Cook J, Peng KWW, Witzig TE, Broski SM, Villasboas JC, Paludo J, *et al.* Clinical Activity of Single Dose Systemic Oncolytic VSV Virotherapy in Patients with Relapsed Refractory T-Cell Lymphoma. *Blood Advances* **2022**;6:3268-79
32. Bishnoi S, Tiwari R, Gupta S, Byrareddy S, Nayak D. Oncotargeting by Vesicular Stomatitis Virus (VSV): Advances in Cancer Therapy. *Viruses* **2018**;10:90-
33. Schirmacher V. Molecular Mechanisms of Anti-Neoplastic and Immune Stimulatory Properties of Oncolytic Newcastle Disease Virus. *Biomedicines* **2022**;10:562-
34. Pol J, Atherton M, Bridle B, Stephenson K, Le Boeuf F, Hummel J, *et al.* Development and applications of oncolytic Maraba virus vaccines. *Oncolytic Virotherapy* **2018**;Volume 7:117-28
35. Tanaka W, Komabayashi K, Ikeda Y, Aoki Y, Itagaki T, Mizuta K. Seroprevalence of coxsackievirus A21 neutralizing antibodies in Yamagata, Japan, between 1976 and 2019; coxsackievirus A21 has rarely affected young children. *Journal of Medical Virology* **2022**;94:2877-81
36. Andtbacka RHI, Curti B, Daniels GA, Hallmeyer S, Whitman ED, Lutzky J, *et al.* Clinical Responses of Oncolytic Coxsackievirus A21 (V937) in Patients With Unresectable Melanoma. *Journal of Clinical Oncology* **2021**;39:3829-38
37. Bradley S, Jakes AD, Harrington K, Pandha H, Melcher A, Errington-Mais F. Applications of coxsackievirus A21 in oncology. *Oncolytic virotherapy* **2014**;3:47-55
38. Li J, Zhang Z, Zhang H, Li M, Li X, Lu L, *et al.* Seroprevalence of poliovirus antibodies before and after polio vaccine switch in 2012 and 2017 in Beijing. *Human Vaccines & Immunotherapeutics* **2021**;17:389-96
39. Díaz-Quiñónez JA, Díaz-Ortega JL, Cruz-Hervert P, Ferreira-Guerrero E, Delgado-Sánchez G, Ferreyra-Reyes L, *et al.* Seroprevalence of Poliomyelitis Antibodies Among Children Aged 1 to 4 Years Old and Factors Associated With Poliovirus Susceptibility; Mexican Health and Nutrition Survey, 2012. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2018**;67:S110-s4

40. Alfonso VH, Voorman A, Hoff NA, Weldon WC, Gerber S, Gadoth A, *et al.* Poliovirus immunity among adults in the Democratic Republic of the Congo: a cross-sectional serosurvey. *BMC Infectious Diseases* **2022**;22:30
41. Kemp V, van den Wollenberg DJM, Camps MGM, van Hall T, Kinderman P, Pronk-van Montfoort N, *et al.* Arming oncolytic reovirus with GM-CSF gene to enhance immunity. *Cancer Gene Therapy* **2018**
42. Groeneveldt C, van Hall T, van der Burg SH, Ten Dijke P, van Montfoort N. Immunotherapeutic Potential of TGF- $\beta$  Inhibition and Oncolytic Viruses. *Trends Immunol* **2020**;41:406-20
43. Kooti W, Esmaeili Gouvarchin Ghaleh H, Farzanehpour M, Dorostkar R, Jalali Kondori B, Bolandian M. Oncolytic Viruses and Cancer, Do You Know the Main Mechanism? *Front Oncol* **2021**;11
44. Mullen JT, Tanabe KK. Viral Oncolysis. *The Oncologist* **2002**;7:106-19
45. Lemos de Matos A, Franco LS, McFadden G. Oncolytic Viruses and the Immune System: The Dynamic Duo. *Molecular Therapy - Methods & Clinical Development* **2020**;17:349-58
46. Russell L, Peng KW, Russell SJ, Diaz RM. Oncolytic Viruses: Priming Time for Cancer Immunotherapy. *BioDrugs* **2019**;33:485-501
47. de Graaf JF, de Vor L, Fouchier RAM, van den Hoogen BG. Armed oncolytic viruses: A kick-start for anti-tumor immunity. *Cytokine & Growth Factor Reviews* **2018**;41:28-39
48. Nguyen T, Avci NG, Shin DH, Martinez-Velez N, Jiang H. Tune Up In Situ Autovaccination against Solid Tumors with Oncolytic Viruses. *Cancers* **2018**;10:171
49. Russell SJ, Barber GN. Oncolytic Viruses as Antigen-Agnostic Cancer Vaccines. *Cancer cell* **2018**;33:599-605
50. Feola S, Russo S, Martins B, Lopes A, Vandermeulen G, Fluhler V, *et al.* Peptides-Coated Oncolytic Vaccines for Cancer Personalized Medicine. *Frontiers in Immunology* **2022**;13:1-16
51. Capasso C, Hirvonen M, Garofalo M, Romaniuk D, Kuryk L, Sarvela T, *et al.* Oncolytic adenoviruses coated with MHC-I tumor epitopes increase the antitumor immunity and efficacy against melanoma. *Oncoimmunology* **2016**;5:e1105429
52. Groeneveldt C, Kinderman P, van den Wollenberg DJM, van den Oever RL, Middelburg J, Mustafa DAM, *et al.* Preconditioning of the tumor microenvironment with oncolytic reovirus converts CD3-bispecific antibody treatment into effective immunotherapy. *J Immunother Cancer* **2020**;8:e001191
53. Groeneveldt C, Kinderman P, van Stigt Thans JJC, Labrie C, Griffioen L, Sluiter M, *et al.* Preinduced reovirus-specific T-cell immunity enhances the anticancer efficacy of reovirus therapy. *Journal for ImmunoTherapy of Cancer* **2022**;10:e004464
54. Shin DH, Nguyen T, Ozpolat B, Lang F, Alonso M, Gomez-Manzano C, *et al.* Current strategies to circumvent the antiviral immunity to optimize cancer virotherapy. *Journal for ImmunoTherapy of Cancer* **2021**;9:e002086-e
55. ClinicalTrials.gov. A Study of Intratumoral CAVATAK™ in Patients With Stage IIIc and Stage IV Malignant Melanoma (VLA-007 CALM). 2010.
56. Kaufman HL, Bommareddy PK. Two roads for oncolytic immunotherapy development. *Journal for ImmunoTherapy of Cancer* **2019**;7:26
57. Andtbacka RHI, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, *et al.* Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. *Journal of Clinical Oncology* **2015**;33:2780-8
58. Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RHI, Michelin O, *et al.* Oncolytic Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. *Cell* **2017**;170:1109-19 e10
59. Bourgeois-Daigneault MC, Roy DG, Aitken AS, El Sayes N, Martin NT, Varette O, *et al.* Neoadjuvant oncolytic virotherapy before surgery sensitizes triple-negative breast cancer to immune checkpoint therapy. *Science translational medicine* **2018**;10

60. Thurber GM, Schmidt MM, Wittrup KD. Antibody tumor penetration: Transport opposed by systemic and antigen-mediated clearance. *Advanced Drug Delivery Reviews* **2008**;60:1421-34
61. Hooks JJ, Burns W, Hayashi K, Geis S, Notkins AL. Viral spread in the presence of neutralizing antibody: mechanisms of persistence in foamy virus infection. *Infection and Immunity* **1976**;14:1172-8
62. Simmons A, Nash AA. Role of antibody in primary and recurrent herpes simplex virus infection. *J Virol* **1985**;53:944-8
63. Bramson JL, Hitt M, Gaudie J, Graham FL. Pre-existing immunity to adenovirus does not prevent tumor regression following intratumoral administration of a vector expressing IL-12 but inhibits virus dissemination. *Gene Therapy* **1997**;4:1069-76
64. Dhar D, Spencer JF, Toth K, Wold WSM. Effect of Preexisting Immunity on Oncolytic Adenovirus Vector INGN 007 Antitumor Efficacy in Immunocompetent and Immunosuppressed Syrian Hamsters. *J Virol* **2009**;83:2130-9
65. Herrlinger U, Kramm CM, Aboody-Guterman KS, Silver JS, Ikeda K, Johnston KM, *et al.* Pre-existing herpes simplex virus 1 (HSV-1) immunity decreases, but does not abolish, gene transfer to experimental brain tumors by a HSV-1 vector. *Gene Therapy* **1998**;5:809-19
66. Li X, Wang P, Li H, Du X, Liu M, Huang Q, *et al.* The Efficacy of Oncolytic Adenovirus Is Mediated by T-cell Responses against Virus and Tumor in Syrian Hamster Model. *Clinical Cancer Research* **2017**;23:239-49
67. Chahlavi A, Rabkin SD, Todo T, Sundaresan P, Martuza RL. Effect of prior exposure to herpes simplex virus 1 on viral vector-mediated tumor therapy in immunocompetent mice. *Gene Therapy* **1999**;6:1751-8
68. Lambright ES, Kang EH, Force S, Lanuti M, Caparrelli D, Kaiser LR, *et al.* Effect of Preexisting Anti-Herpes Immunity on the Efficacy of Herpes Simplex Viral Therapy in a Murine Intraperitoneal Tumor Model. *Molecular Therapy* **2000**;2:387-93
69. Burnett WJ, Burnett DM, Parkman G, Ramstead A, Contreras N, Gravley W, *et al.* Prior Exposure to Coxsackievirus A21 Does Not Mitigate Oncolytic Therapeutic Efficacy. *Cancers* **2021**;13:4462-
70. Davola ME, Mossman KL. Oncolytic viruses: how “lytic” must they be for therapeutic efficacy? *Oncol Immunology* **2019**;8:e1581528
71. Annelis NE, Mansfield D, Arif M, Ballesteros-Merino C, Simpson GR, Denyer M, *et al.* Phase I Trial of an ICAM-1-Targeted Immunotherapeutic-Coxsackievirus A21 (CVA21) as an Oncolytic Agent Against Non Muscle-Invasive Bladder Cancer. *Clinical Cancer Research* **2019**;25:5818-31
72. Andtbacka RHI, Curti BD, Kaufman H, Daniels GA, Nemunaitis JJ, Spitler LE, *et al.* Final data from CALM: A phase II study of Coxsackievirus A21 (CVA21) oncolytic virus immunotherapy in patients with advanced melanoma. *Journal of Clinical Oncology* **2015**;33:9030-
73. Andtbacka RHI, Ross MI, Agarwala SS, Taylor MH, Vetto JT, Neves RI, *et al.* Final results of a phase II multicenter trial of HF10, a replication-competent HSV-1 oncolytic virus, and ipilimumab combination treatment in patients with stage IIIB-IV unresectable or metastatic melanoma. *Journal of Clinical Oncology* **2017**;35:9510-
74. Bazan-Peregrino M, Garcia-Carbonero R, Laquente B, Álvarez R, Mato-Berciano A, Gimenez-Alejandre M, *et al.* VCN-01 disrupts pancreatic cancer stroma and exerts antitumor effects. *Journal for ImmunoTherapy of Cancer* **2021**;9:e003254-e
75. Kirn DH, Wang Y, Liang W, Contag CH, Thorne SH. Enhancing Poxvirus Oncolytic Effects through Increased Spread and Immune Evasion. *Cancer Research* **2008**;68:2071-5
76. Ferguson MS, Lemoine NR, Wang Y. Systemic Delivery of Oncolytic Viruses: Hopes and Hurdles. *Advances in Virology* **2012**;2012:1-14
77. Miest TS, Yaiw K-C, Frenzke M, Lampe J, Hudacek AW, Springfield C, *et al.* Envelope-chimeric Entry-targeted Measles Virus Escapes Neutralization and Achieves Oncolysis. *Molecular Therapy* **2011**;19:1813-20

78. Xia M, Luo D, Dong J, Zheng M, Meng G, Wu J, *et al.* Graphene oxide arms oncolytic measles virus for improved effectiveness of cancer therapy. *Journal of Experimental & Clinical Cancer Research* **2019**;38:408-
79. Bah ES, Nace RA, Peng KW, Muñoz-Alía MÁ, Russell SJ. Retargeted and Stealth-Modified Oncolytic Measles Viruses for Systemic Cancer Therapy in Measles Immune Patients. *Mol Cancer Ther* **2020**;19:2057-67
80. Chen Y, Yu D-C, Charlton D, Henderson DR. Pre-Existent Adenovirus Antibody Inhibits Systemic Toxicity and Antitumor Activity of CN706 in the Nude Mouse LNCaP Xenograft Model: Implications and Proposals for Human Therapy. *Human Gene Therapy* **2000**;11:1553-67
81. Power AT, Wang J, Falls TJ, Paterson JM, Parato KA, Lichty BD, *et al.* Carrier Cell-based Delivery of an Oncolytic Virus Circumvents Antiviral Immunity. *Molecular Therapy* **2007**;15:123-30
82. Delman KA, Bennett JJ, Zager JS, Burt BM, McAuliffe PF, Petrowsky H, *et al.* Effects of Preexisting Immunity on the Response to Herpes Simplex-Based Oncolytic Viral Therapy. *Human Gene Therapy* **2000**;11:2465-72
83. Adair RA, Roulstone V, Scott KJ, Morgan R, Nuovo GJ, Fuller M, *et al.* Cell Carriage, Delivery, and Selective Replication of an Oncolytic Virus in Tumor in Patients. *Science Translational Medicine* **2012**;4
84. Ilett EJ, Bárcena M, Errington-Mais F, Griffin S, Harrington KJ, Pandha HS, *et al.* Internalization of Oncolytic Reovirus by Human Dendritic Cell Carriers Protects the Virus from Neutralization. *Clinical Cancer Research* **2011**;17:2767-76
85. Ilett E, Kottke T, Donnelly O, Thompson J, Willmon C, Diaz R, *et al.* Cytokine Conditioning Enhances Systemic Delivery and Therapy of an Oncolytic Virus. *Molecular Therapy* **2014**;22:1851-63
86. Ilett EJ, Prestwich RJ, Kottke T, Errington F, Thompson JM, Harrington KJ, *et al.* Dendritic cells and T cells deliver oncolytic reovirus for tumour killing despite pre-existing anti-viral immunity. *Gene Therapy* **2009**;16:689-99
87. Ong HT, Hasegawa K, Dietz AB, Russell SJ, Peng KW. Evaluation of T cells as carriers for systemic measles virotherapy in the presence of antiviral antibodies. *Gene Therapy* **2007**;14:324-33
88. Zhou Y-C, Zhang Y-N, Yang X, Wang S-B, Hu P-Y. Delivery systems for enhancing oncolytic adenoviruses efficacy. *International Journal of Pharmaceutics* **2020**;591:119971-
89. Batalla-Covello J, Ngai HW, Flores L, McDonald M, Hyde C, Gonzaga J, *et al.* Multiple Treatment Cycles of Neural Stem Cell Delivered Oncolytic Adenovirus for the Treatment of Glioblastoma. *Cancers* **2021**;13:6320-
90. Berkeley RA, Steele LP, Mulder AA, van den Wollenberg DJM, Kottke TJ, Thompson J, *et al.* Antibody-Neutralized Reovirus Is Effective in Oncolytic Virotherapy. *Cancer Immunology Research* **2018**;6:1161-73
91. Leopold PL, Wendland RL, Vincent T, Crystal RG. Neutralized Adenovirus-Immune Complexes Can Mediate Effective Gene Transfer via an Fc Receptor-Dependent Infection Pathway. *J Virol* **2006**;80:10237-47
92. Yang X, Zhang X, Zhao X, Yuan M, Zhang K, Dai J, *et al.* Antibody-Dependent Enhancement: "Evil" Antibodies Favorable for Viral Infections. *Viruses* **2022**;14
93. Mok DZL, Chan KR. The Effects of Pre-Existing Antibodies on Live-Attenuated Viral Vaccines. *Viruses* **2020**;12:520
94. Montagnaro S, Damiano S, Ciarcia R, Puzio MV, Ferrara G, Iovane V, *et al.* Caprine herpesvirus 1 (CpHV-1) as a potential candidate for oncolytic virotherapy. *Cancer Biol Ther* **2019**;20:42-51
95. Forte IM, Indovina P, Montagnaro S, Costa A, Iannuzzi CA, Capone F, *et al.* The Oncolytic Caprine Herpesvirus 1 (CpHV-1) Induces Apoptosis and Synergizes with Cisplatin in Mesothelioma Cell Lines: A New Potential Virotherapy Approach. *Viruses* **2021**;13:2458-

96. Bots STF, Kemp V, Cramer SJ, van den Wollenberg DJM, Hornsveld M, Lamfers MLM, *et al.* Nonhuman Primate Adenoviruses of the Human Adenovirus B Species Are Potent and Broadly Acting Oncolytic Vector Candidates. *Human Gene Therapy* **2022**;33:275-89
97. Kozak R, Hattin L, Biondi M, Corredor J, Walsh S, Xue-Zhong M, *et al.* Replication and Oncolytic Activity of an Avian Orthoreovirus in Human Hepatocellular Carcinoma Cells. *Viruses* **2017**;9:90-
98. Koppers-Lalic D, Hoeben RC. Non-human viruses developed as therapeutic agent for use in humans. *Rev Med Virol* **2011**;21:227-39
99. Hoeben RC, Louz D, Koppers-Lalic D. Biosafety of non-human therapeutic viruses in clinical gene therapy. *Curr Gene Ther* **2013**;13:492-9
100. Tuzmen C, Cairns TM, Atanasiu D, Lou H, Saw WT, Hall BL, *et al.* Point Mutations in Retargeted gD Eliminate the Sensitivity of EGFR/EGFRvIII-Targeted HSV to Key Neutralizing Antibodies. *Molecular Therapy - Methods & Clinical Development* **2020**;16:145-54
101. Neault S, Bossow S, Achard C, Bell JC, Diallo JS, Leber MF, *et al.* Robust envelope exchange platform for oncolytic measles virus. *Journal of Virological Methods* **2022**;302:114487-
102. Roberts DM, Nanda A, Havenga MJE, Abbink P, Lynch DM, Ewald BA, *et al.* Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. *Nature* **2006**;441:239-43
103. Zafar S, Quixabeira DCA, Kudling TV, Cervera-Carrascon V, Santos JM, Grönberg-Vähä-Koskela S, *et al.* Ad5/3 is able to avoid neutralization by binding to erythrocytes and lymphocytes. *Cancer Gene Therapy* **2021**;28:442-54
104. Stepanenko AA, Sosnovtseva AO, Valikhov MP, Chernysheva AA, Cherepanov SA, Yusubalieva GM, *et al.* Superior infectivity of the fiber chimeric oncolytic adenoviruses Ad5/35 and Ad5/3 over Ad5-delta-24-RGD in primary glioma cultures. *Molecular Therapy - Oncolytics* **2022**;24:230-48
105. Rojas LA, Condezo GN, Moreno R, Fajardo CA, Arias-Badia M, San Martín C, *et al.* Albumin-binding adenoviruses circumvent pre-existing neutralizing antibodies upon systemic delivery. *Journal of Controlled Release* **2016**;237:78-88
106. Naito T, Kaneko Y, Kozbor D. Oral vaccination with modified vaccinia virus Ankara attached covalently to TMPEG-modified cationic liposomes overcomes pre-existing poxvirus immunity from recombinant vaccinia immunization. *Journal of General Virology* **2007**;88:61-70
107. Nosaki K, Hamada K, Takashima Y, Sagara M, Matsumura Y, Miyamoto S, *et al.* A novel, polymer-coated oncolytic measles virus overcomes immune suppression and induces robust antitumor activity. *Molecular Therapy - Oncolytics* **2016**;3:16022-
108. Huang H, Sun M, Liu M, Pan S, Liu P, Cheng Z, *et al.* Full encapsulation of oncolytic virus using hybrid erythrocyte-liposome membranes for augmented anti-refractory tumor effectiveness. *Nano Today* **2022**;47:101671
109. Tysome JR, Li X, Wang S, Wang P, Gao D, Du P, *et al.* A Novel Therapeutic Regimen to Eradicate Established Solid Tumors with an Effective Induction of Tumor-Specific Immunity. *Clinical Cancer Research* **2012**;18:6679-89
110. Irvine KR, Chamberlain RS, Shulman EP, Surman DR, Rosenberg SA, Restifo NP. Enhancing Efficacy of Recombinant Anticancer Vaccines With Prime/Boost Regimens That Use Two Different Vectors. *JNCI Journal of the National Cancer Institute* **1997**;89:1595-601
111. Fazekas de St.Groth S, Webster RG. Disquisitions on Original Antigenic Sin. *Journal of Experimental Medicine* **1966**;124:331-45
112. Zhang A, Stacey HD, Mullarkey CE, Miller MS. Original Antigenic Sin: How First Exposure Shapes Lifelong Anti-Influenza Virus Immune Responses. *J Immunol* **2019**;202:335-40
113. Shirley JL, de Jong YP, Terhorst C, Herzog RW. Immune Responses to Viral Gene Therapy Vectors. *Mol Ther* **2020**;28:709-22
114. Schirmbeck R, Reimann J, Kochanek S, Kreppel F. The Immunogenicity of Adenovirus Vectors Limits the Multispecificity of CD8 T-cell Responses to Vector-encoded Transgenic Antigens. *Molecular Therapy* **2008**;16:1609-16

115. Altenburg AF, van Trierum SE, de Bruin E, de Meulder D, van de Sandt CE, van der Klis FRM, *et al.* Effects of pre-existing orthopoxvirus-specific immunity on the performance of Modified Vaccinia virus Ankara-based influenza vaccines. *Scientific Reports* **2018**;8:6474-
116. Frahm N, DeCamp AC, Friedrich DP, Carter DK, Defawe OD, Kublin JG, *et al.* Human adenovirus-specific T cells modulate HIV-specific T cell responses to an Ad5-vectored HIV-1 vaccine. *Journal of Clinical Investigation* **2012**;122:359-67
117. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, *et al.* Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *The Lancet* **2008**;372:1881-93
118. Ledgerwood JE, Costner P, Desai N, Holman L, Enama ME, Yamshchikov G, *et al.* A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. *Vaccine* **2010**;29:304-13
119. Pol JG, Zhang L, Bridle BW, Stephenson KB, Rességuier J, Hanson S, *et al.* Maraba Virus as a Potent Oncolytic Vaccine Vector. *Molecular Therapy* **2014**;22:420-9
120. ClinicalTrials.gov. MG1 Maraba/MAGE-A3, With and Without Adenovirus Vaccine With Transgenic MAGE-A3 Insertion in Incurable MAGE-A3-Expressing Solid Tumours - Full Text View - ClinicalTrials.gov. 2014.
121. ClinicalTrials.gov. Oncolytic MG1-MAGEA3 With Ad-MAGEA3 Vaccine in Combination With Pembrolizumab for Non-Small Cell Lung Cancer Patients - Full Text View - ClinicalTrials.gov. 2016.
122. Ricca JM, Oseledchik A, Walther T, Liu C, Mangarin L, Merghoub T, *et al.* Pre-existing Immunity to Oncolytic Virus Potentiates Its Immunotherapeutic Efficacy. *Molecular Therapy* **2018**;26:1008-19
123. Ding J, Murad YM, Sun Y, Lee IF, Samudio I, Liu X, *et al.* Pre-Existing HSV-1 Immunity Enhances Anticancer Efficacy of a Novel Immune-Stimulating Oncolytic Virus. *Viruses* **2022**;14:2327-
124. Borst J, Ahrends T, Bąbala N, Melief CJM, Kastenmüller W. CD4+ T cell help in cancer immunology and immunotherapy. *Nature Reviews Immunology* **2018**;18:635-47
125. Kumamoto Y, Mattei LM, Sellers S, Payne GW, Iwasaki A. CD4 + T cells support cytotoxic T lymphocyte priming by controlling lymph node input. *Proceedings of the National Academy of Sciences* **2011**;108:8749-54
126. Tähtinen S, Feola S, Capasso C, Laustio N, Groeneveldt C, Ylösmäki EO, *et al.* Exploiting Preexisting Immunity to Enhance Oncolytic Cancer Immunotherapy. *Cancer Research* **2020**;80:2575-85
127. Brown MC, McKay ZP, Yang Y, Beasley GM, Ashley DM, Bigner DD, *et al.* Intratumor Childhood Vaccine-Specific CD4+ T cell Recall Coordinates Antitumor CD8+ T cells and Eosinophils. *bioRxiv* **2022**:2022.03.14.484260
128. Rosato PC, Wijeyesinghe S, Stolley JM, Nelson CE, Davis RL, Manlove LS, *et al.* Virus-specific memory T cells populate tumors and can be repurposed for tumor immunotherapy. *Nature Communications* **2019**;10:567
129. Simoni Y, Becht E, Fehlings M, Loh CY, Koo SL, Teng KWW, *et al.* Bystander CD8+ T cells are abundant and phenotypically distinct in human tumour infiltrates. *Nature* **2018**;557:575-9
130. Cao D, Song Q, Li J, Chard Dunmall LS, Jiang Y, Qin B, *et al.* Redirecting anti-Vaccinia virus T cell immunity for cancer treatment by AAV-mediated delivery of the VV B8R gene. *Molecular Therapy - Oncolytics* **2022**;25:264-75
131. Çuburu N, Bialkowski L, Pontejo SM, Sethi SK, Bell ATF, Kim R, *et al.* Harnessing anti-cytomegalovirus immunity for local immunotherapy against solid tumors. *Proc Natl Acad Sci U S A* **2022**;119:e2116738119
132. Millar DG, Ramjiawan RR, Kawaguchi K, Gupta N, Chen J, Zhang S, *et al.* Antibody-mediated delivery of viral epitopes to tumors harnesses CMV-specific T cells for cancer therapy. *Nature Biotechnology* **2020**;38:420-5



133. Sefrin JP, Hillringhaus L, Mundigl O, Mann K, Ziegler-Landesberger D, Seul H, *et al.* Sensitization of Tumors for Attack by Virus-Specific CD8+ T-Cells Through Antibody-Mediated Delivery of Immunogenic T-Cell Epitopes. **2019**;10
134. Jung K, Son M-J, Lee S-Y, Kim J-A, Ko D-H, Yoo S, *et al.* Antibody-mediated delivery of a viral MHC-I epitope into the cytosol of target tumor cells repurposes virus-specific CD8+ T cells for cancer immunotherapy. *Molecular Cancer* **2022**;21:102
135. Chiaro J, Kasanen HHE, Whalley T, Capasso C, Grönholm M, Feola S, *et al.* Viral Molecular Mimicry Influences the Antitumor Immune Response in Murine and Human Melanoma. *Cancer Immunol Res* **2021**;9:981-93
136. Chaganty BKR, Qiu S, Lu Y, Lopez-Berestein G, Ozpolat B, Fan Z. Redirecting host preexisting influenza A virus immunity for cancer immunotherapy. *Cancer Immunology, Immunotherapy* **2022**;71:1611-23
137. Aznar MA, Molina C, Teixeira A, Rodriguez I, Azpilikueta A, Garasa S, *et al.* Repurposing the yellow fever vaccine for intratumoral immunotherapy. *EMBO Mol Med* **2020**;12:1-17
138. Gujar S, Pol JG, Kim Y, Kroemer G. Repurposing CD8+ T cell immunity against SARS-CoV-2 for cancer immunotherapy: a positive aspect of the COVID-19 pandemic? *Oncoimmunology* **2020**;9:1794424
139. Ordóñez-Reyes C, García-Robledo JE, Chamorro DF, Mosquera A, Sussmann L, Ruiz-Patiño A, *et al.* Bispecific Antibodies in Cancer Immunotherapy: A Novel Response to an Old Question. *Pharmaceutics* **2022**;14:1243-
140. Conti M. Boosting effect of pre-existing immunity on anti-cancer immunotherapies. *Frontiers in Drug, Chemistry and Clinical Research* **2021**;4:1-6
141. Niemann J, Woller N, Brooks J, Fleischmann-Mundt B, Martin NT, Kloos A, *et al.* Molecular retargeting of antibodies converts immune defense against oncolytic viruses into cancer immunotherapy. *Nature Communications* **2019**;10:3236-
142. Pinto S, Pahl J, Schottelius A, Carter PJ, Koch J. Reimagining antibody-dependent cellular cytotoxicity in cancer: the potential of natural killer cell engagers. *Trends in Immunology* **2022**;43:932-46
143. Heidbuechel JPW, Engeland CE. Oncolytic viruses encoding bispecific T cell engagers: a blueprint for emerging immunovirotherapies. *Journal of Hematology & Oncology* **2021**;14:63
144. Rosenbaum P, Tchitchek N, Joly C, Rodríguez Pozo A, Stimmer L, Langlois S, *et al.* Vaccine Inoculation Route Modulates Early Immunity and Consequently Antigen-Specific Immune Response. *Frontiers in Immunology*. Volume 122021. p 645210.



