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Virus nanotechnology for intratumoural immunotherapy

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Abstract

Viruses can be designed to be tools and carrier vehicles for intratumoural immunotherapy. Their nanometre-scale size and shape allow for functionalization with or encapsulation of medical cargoes and tissue-specific ligands. Importantly, immunotherapies may particularly benefit from the inherent immunomodulatory properties of viruses. For example, mammalian viruses have already been tested for oncolytic virotherapy, and bacteriophages and plant viruses can be engineered for immunotherapeutic treatment approaches. In this Review, we discuss how viruses

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N.F.S. and A.O.O. conceived and developed the topic. All authors contributed to the writing, editing and proof-reading of the manuscript.

Competing interests

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— including oncolytic viruses, immunomodulatory plant viruses and bacteriophages — and virus-like particles can be designed for intratumoural immunotherapy to elicit anti-tumour immunity and induce systemic anti-tumour responses at distant non-injected sites. We further highlight the engineering of viruses and virus-like particles as drug-delivery systems, and outline key translational challenges and clinical opportunities.

Introduction

Immunotherapy is a new addition to long-established cancer treatment strategies, such as surgery, radiotherapy and chemotherapy¹. Immunotherapy trains the patients' immune system to recognize and eradicate cancer cells systemically². Several immunotherapies have been approved by the FDA or are in clinical development, including immune checkpoint inhibitors (such as cytotoxic T-lymphocyte-associated antigen 4, CTLA-4 (ref. 3); programmed cell death 1 and its ligand, PD1–PDL1; and lymphocyte activation gene 3, Lag3), tumour vaccines (such as sipuleucel-T), cell-based immunotherapies (such as chimeric antigen receptor (CAR)-T-cell therapy⁴) and small-molecule-based immunotherapies (such as stimulator of interferon genes protein (STING) agonists)⁵. We note that the development of the FDA-approved drugs CTLA-4 and PD1–PDL1 was awarded the Nobel prize in 2018 (ref. 6). In addition, viruses are explored for immunotherapy owing to their intrinsic immunomodulatory features, because they can evade and/or generate immune responses in their hosts through structural recognition motifs and other molecular mechanisms⁷. Thereby, viruses can interface with and reprogram the immune system and thus be repurposed for immunotherapy^{8–10}.

Our current understanding of the relation between the immune system and cancer is reflected by the cancer-immunity cycle^{11,12} (Fig. 1). Many factors contribute to tumour-mediated immune suppression and cancer progression and are targets for therapeutic intervention¹³. These factors include cancer-cell-surface receptors and secreted factors, matrix stiffness, interstitial pressure, hypoxia, low intratumoural pH, leaky vasculature, limited drainage to lymph nodes and prevention of infiltration by effector T cells and other immune cells into the tumour microenvironment (TME)¹³. Most tumours recruit and polarize immunosuppressive immune cells within the TME to generate local tumour-supporting immune suppression¹². Activation of the cancer-immunity cycle, which is limited by this immunosuppressive environment, is the primary goal of immunotherapy. In particular, stimulation of the type I interferon (IFN) pathway is being investigated as a therapeutic strategy. Although type I IFN signalling can have anti-neoplastic effects, it is primarily (but not only) an antiviral response^{14,15}. Upon pathogenic insult and recognition, local immune cells are alerted to secrete type I IFN¹⁶.

Type I IFNs reprogram the TME through autocrine and paracrine circuits, which leads to the upregulation of IFN-stimulated genes and activation of the anti-pathogenic state of immune cells¹⁴. Specifically, type I IFN programs upregulate antigen presentation by dendritic cells, enhance the cytotoxicity of CD8⁺ T cells and natural killer cells, polarize macrophages toward inflammatory phenotypes and reduce the immunosuppressive state of regulatory T

cells^{14,16}. In addition, type I IFN promotes crosstalk that stimulates the adaptive immune system and establishes B- and T-cell-mediated antigen-specific memory¹⁶.

Stimulation of the type I IFN pathway also holds promise in cancer immunotherapy, and recombinant IFN α was one of the first approved cancer immunotherapy drugs¹⁷. However, recombinant IFN α has a short half-life in serum, and therefore treatment with recombinant IFN α requires high dosage through intravenous or subcutaneous injections of up to five times a week for extended periods^{18,19}. Viruses can be repurposed as drugs to produce type I IFNs, enabling sustained IFN α levels in particular, because viruses and their nanoparticle formulations typically have good tissue residence and viral replication may further extend IFN α signalling. Thus, virus-based immunomodulation may allow the reduction of dosage, and hence the costs and infrastructural burden of IFN α immunotherapy. Several virus-based drug candidates that target the type I IFN signalling pathway are under development, including PVSRIPO (a modified poliovirus:rhinovirus chimera)²⁰, vidutolimod (a bacteriophage virus-like particle (VLP) carrying TLR9 receptor agonists)²¹, and plant viruses, such as papaya mosaic virus²² and cowpea mosaic virus²³.

In this Review, we discuss how virus nanotechnology can be designed to activate the cancer-immunity cycle. In particular, we examine the application of oncolytic viruses, which selectively replicate in and lyse tumour cells, non-infectious plant viruses, which can agonize the mammalian immune system, and virus-delivery systems, including plant virus- and bacteriophage-derived VLPs (which are not infectious to their hosts because they are devoid of genomic nucleic acid²⁴), for intratumoural immunotherapy.

Intratumoural immunotherapy

The efficacy of intratumoural therapeutic delivery was first demonstrated by administering bacteria to tumours and surgical tumour sites, resulting in the local reduction in tumour growth in human patients^{25,26} (Fig. 2). Importantly, this therapeutic strategy can also reduce or eliminate distant untreated tumours. The idea that the immune system protects against cancer was proposed around 1909 (ref. 27), but the concept was only later developed in the late 1950s to early 1970s^{28,29} and is now known as cancer immunosurveillance. Various intratumoural immunotherapies have since been developed and approved, such as talimogene laherparepvec (TVEC)³⁰. Intratumoural immunotherapy primarily acts on innate immune cells (such as dendritic cells, natural killer cells and macrophages) to rewire the TME and relieve local immunosuppression, which leads to crosstalk with adaptive immune cells (CD4⁺ and CD8⁺ T cells) to induce systemic immune-mediated tumour-cell death³¹. Compared to immunotherapy by systemic intravenous injections³⁰, intratumoural immunotherapy achieves higher drug concentration at the tumour site, while considerably reducing systemic drug exposure, translating to increased safety and reduced costs^{32–34}. Furthermore, host-immune responses can augment or compromise clinical drug efficacy after systemic administration^{30,35}. For example, neutralizing anti-drug antibodies are a barrier for systemic delivery of oncolytic virotherapies³², and non-neutralizing anti-drug antibodies can alter the biodistribution and pharmacokinetics of biotherapeutics³⁶, which may be addressed by intratumoural immunotherapy.

However, intratumoural immunotherapy of large tumours requires multiple injections, leading to procedural complexities and efficacy variance. In addition, high intra-tumoural fluid pressure may prevent effective drug dispersion or cause the exit of drugs from the tumour³⁷. Recurrent dosing may further affect patient compliance owing to discomfort³⁸. Material and treatment designs, such as slow-release depots³⁹, may be able to address these issues by stream-lining intratumoural administration, thereby alleviating the need for repeated treatment.

Virus nanotechnology

Virus nanotechnology refers to the repurposing of viruses and VLPs, that is, assembled virus particles without genomic content, for nanotechnology approaches. In particular, since its discovery in the 1890s as ‘contagium vivum fluidum’, the rod-shaped tobacco mosaic virus (TMV)^{40,41} has been used as a tool for virus nanotechnology⁴² (Fig. 2). In 1939, TMV was the first virus to be imaged using an electron microscope, greatly advancing the field of virology⁴³. The structural principles and triangulation numbers of icosahedral-shaped viruses were described in the early 1960s⁴⁴. In addition, structure-based engineering has been applied to create virus-based vaccines, and concepts such as genetic overcoat display (that is, display of proteins of interest on virus capsids through genetic engineering), encapsulation and bioconjugation have been developed for icosahedral-shaped viruses^{45,46}. Viruses and VLPs are biological nanoscale materials that offer a design space for versatile applications, including drug and gene delivery^{47,48}, light harvesting^{49,50}, data and energy storage^{51,52} and nanobiocatalysis^{53,54}. Viruses have also been engineered for medical applications, with the first DNA recombinant VLP vaccine approved by the FDA for hepatitis B in 1986 (refs. 55,56) (Fig. 2). The first human gene therapy using recombinant adenovirus was approved in 1993 (refs. 57,58), and replication-competent and -incompetent viruses are now being explored for cancer immunotherapy^{59,60}.

Multiple pathways are involved in cancer progression⁶¹, and therefore, treatment approaches may benefit from viruses owing to their multi-mechanistic actions. In particular, the nanoscale size of viruses and VLPs enables tissue retention, delivery and protection of cargo, cell engagement and lymphatic drainage^{24,62}. In addition, the highly ordered and repetitive arrangement of viral protein capsids serves as a pathogen-associated molecular pattern (PAMP) that can generate a response from immune cells^{63,64}. Nucleoprotein assemblies may further contain multiple factors that activate the immune system; for example, nucleic acid sequences can target Toll-like receptors (TLRs), T helper (T_H) cell epitopes or carbohydrates that stimulate T cells⁶⁴. However, translating virus nanotechnology for cancer immunotherapy requires an understanding of how viral features, such as nucleic acids, capsids and ligand–receptor binding, can be intentionally harnessed and re-engineered to modulate the TME.

Oncolytic viruses

The increase in our knowledge of virus–host interactions and genetic engineering tools has enabled the development of oncolytic viruses as a cancer treatment modality⁶⁵ (Fig. 3), with four therapies approved for intratumoural immunotherapy. These are ECHO-7

(echovirus, first approved in 2004 in Latvia, discontinued owing to lack of efficacy and manufacturing issues)⁶⁶, H101 (adenovirus, approved in 2005 in China)⁶⁷, TVEC (herpes simplex virus type 1 (HSV1), approved in 2015 in the USA)⁶⁰, and Teserpaturev (HSV1, approved in 2021 in Japan)⁶⁸. Oncolytic viruses are engineered to target, infect and lyse cancerous cells⁶⁹, which causes the release of tumour-associated antigens and neoantigens (Fig. 4a). Viral replication and the expression of foreign, immunogenic viral proteins also lead to the release of proinflammatory cytokines and chemokines, which causes the recruitment and activation of innate and adaptive immune cells within the TME, resulting in antigen processing and presentation and thus systemic adaptive anti-tumour immunity^{70–73}. To engineer an oncolytic-virus-based immunotherapy, four major parameters need to be considered: optimization of tropism for specific cancer cells; reduction of virulence toward healthy cells; improvement of the immune-stimulatory function of oncolytic viruses; and avoidance of drug neutralization by the host-immune response^{73,74}.

Cancer-cell targeting

Cancer cells can be targeted by exploiting upregulated or aberrant expression of viral receptors, such as CD46 (a membrane cofactor protein)⁷⁵, CD155 (a poliovirus receptor), herpes virus entry mediator (HVEM)⁷⁶, nectin-1 or -2 (herpesvirus entry mediator C or B)^{77,78} and integrins (transmembrane receptors)⁷⁹. Oncolytic viruses can then use these receptors for viral entry; for example, HSV1 enters host cells through interaction with HVEM or nectin-1/-2, a mechanism exploited in TVEC⁸⁰; measles virus binds to CD46 for cell entry⁸¹; and poliovirus enters via CD155 (ref. 82). Moreover, viruses without natural tropism for cancer cells can be genetically engineered to acquire tumour-cell-targeting properties; for example, in the chimeric oncolytic adenovirus 5/3, the Ad5 fibre knob is replaced by the CD46-specific and desmoglein-2-specific Ad3 fibre knob⁸³. In addition, an RGD peptide can be integrated in oncolytic viruses to allow binding to integrin receptors overexpressed on cancer cells, thereby increasing tumour-cell penetration and engagement^{84,85}. An alternative targeting strategy can take advantage of proteases found within the TME, such as matrix metalloproteinase 9 (MMP9). For example, a tuneable adeno-associated virus (AAV) has been developed to be selectively activated only in the presence of MMP9 (ref. 86). These targeting strategies, which can be applied to viruses, biologics or synthetic nanoparticles, have been widely explored in nanomedicine, albeit with limited translational success^{87,88}.

Cancer cells often possess irregular transcriptional and signalling pathways owing to mutations⁸⁹. This not only contributes to their uncontrolled cell replication but may also lead to a compromised antiviral response, such as suppressed type I IFN responses and inhibited cell apoptosis for viral clearance^{90,91}. Therefore, oncolytic viruses can be engineered to preferentially replicate in tumour cells rather than in healthy cells. For example, overexpression of anti-apoptotic B-cell leukaemia/lymphoma 2 (Bcl-2) family proteins can inhibit cancer-cell apoptosis^{92,93}. This can be exploited by engineering a Newcastle disease virus-based oncolytic virus that can replicate in human B-cell lymphoma extra-large (Bcl-xL) over-expressing non-small-cell lung cancer cell line (A549), allowing the spread of infection and thus oncolytic effects⁹⁴. Oncolytic viruses can also be designed to replicate under cancer-specific or tissue-specific control. For example, an oncolytic

adenovirus can be engineered to express the adenoviral E1A protein (which enhances viral replication) under control of the prostate specific antigen (PSA) promoter, thereby achieving selective E1A expression in PSA-expressing human prostate cancer cells and its xenografts in mice⁹⁵. Here, adenoviral E1A protein expression inhibits cell cycle arrest, enabling sufficient virus replication to achieve oncolysis⁹⁶. Although these strategies can be applied to target tumour cells, such engineered oncolytic viruses may also infect healthy cells that express the same tissue-specific promoters. Therefore, tumour and healthy cells should be profiled by proteomics or gene sequencing to delineate signatures that can be specifically targeted.

Immunostimulation

As pathogens, oncolytic viruses are recognized by pathogen recognition receptors (PRRs), which, together with viral replication and protein expression, prime antiviral immune responses, causing the release of pro-inflammatory cytokines, such as type I IFNs. Nevertheless, tumours can have impaired antiviral functions, and thus, transgenes can be incorporated into viruses⁷³ to augment this immune response and reprogramme the TME^{65,97}. For example, genes can be implemented that encode immunostimulatory cytokines and chemokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), the interleukins IL-2, IL-12 and IL-15, and the CXC motif chemokine ligands CXCL9 and CXCL10 (ref. 73). Furthermore, preclinical and clinical studies have shown that treatment with oncolytic viruses can increase the expression of programmed cell death-ligand 1 (PD-L1) in cancer cells⁹⁸. Accordingly, oncolytic viruses can be engineered to express anti-PD-L1^{99,100}. Other immune-activating ligands, such as cluster of differentiation 40 ligand (CD40L), OX40 ligand (OX40L) and the 4-1BB ligand (4-1BBL)^{101–103}, as well as suicide genes (for example, thymidine kinase to activate the prodrug ganciclovir) can be introduced into oncolytic viruses, an approach that has been tested in preclinical and clinical studies^{104–106}. Transgenes can also be introduced in non-oncolytic viruses, including in viral vectors and VLPs. For example, transgenes for human IFN α 2b have been introduced in the FDA-approved therapy nadofaragene firadenovec, a non-replicating adenoviral vector encoding IFN α 2b for bacillus Calmette–Guerin (BCG)-unresponsive, non-muscle-invasive bladder cancer (NMIBC) treatment^{36,107}.

Safety

The clinical translation of oncolytic viruses remains limited by pathogenicity-related safety concerns³⁶ as well as efficacy issues, partly owing to neutralization by pre-existing antiviral immunity (that is, anti-drug antibodies)¹⁰⁸. However, the safety of oncolytic viruses can be improved by deleting or replacing virulence genes. For example, TVEC has a deletion of the γ 34.5 gene encoding the neurovirulence factor ICP34.5 within HSV1^{109,110}, which diminishes its pathogenicity; in the poliovirus-based oncolytic virus PVSRIPO, the original viral internal ribosome entry site sequence is replaced with a sequence from human rhinovirus type 2 to avoid neuron infection¹¹¹.

Anti-drug antibodies

Oncolytic viruses are typically administered through intratumoural injection¹¹² to enable the treatment of recognized tumours and to limit systemic virus exposure and organ damage¹¹³.

However, both intravenous and intratumoural virus administration are challenged by pre-existing or newly established anti-drug antibodies, that is, antibodies developed owing to prior exposure to the virus or in response to repetitive intravenous administration, respectively. This is particularly problematic for Ad5 and HSV1, to which many people have previously been exposed^{114,115}. The presence of anti-drug antibodies leads to clearance of intravenously injected oncolytic viruses, thus limiting their accumulation in tumour sites. Importantly, anti-drug antibodies can also prevent oncolytic viruses from infecting tumour cells following intratumoural injection. To escape from anti-drug antibody-mediated clearance, oncolytic viruses can be coated with polymers¹¹⁶, liposomes¹¹⁷ or graphene sheets¹¹⁸. Alternatively, albumin-binding protein can be genetically inserted on the drug surface, leading to the binding of albumin to viruses instead of antiviral antibodies to prolong circulation and proliferation¹¹⁹. In addition, patient-derived mesenchymal stem cells, neural stem cells or other immune cells can be used as carriers for oncolytic-virus delivery, because viruses loaded in such immune cells can be protected from antiviral antibody recognition and clearance, thereby achieving longer circulation and delivery to tumour sites^{120–122}.

Abscopal effect

The clinical impact of intratumoural immunotherapy depends on its systemic efficacy, that is, the elimination or inhibition of distant, non-injected tumours. This effect, which is termed the ‘abscopal effect’, results from the activation of systemic anti-tumour immunity. The abscopal effect, or a reduction in tumour volume at non-injected distant metastases, has been clinically observed for TVEC¹⁰, V937 (oncolytic coxsackievirus A21)¹²³ and Pexa-Vec (JX-594, oncolytic vaccinia virus)¹²⁴. However, the abscopal effect remains a rare and unpredictable phenomenon, and the underlying mechanisms and how these can be harnessed for cancer treatment remain to be investigated.

Translational challenges

Despite preclinical and clinical efforts^{36,112,125} (Fig. 3; Table 1), challenges remain to be overcome for the wider clinical translation of oncolytic-virus-based intratumoural immunotherapy. In particular, tumour heterogeneity may impede oncolytic virus infection; the presence of anti-drug antibodies may cause virus clearance; and solo treatment with an oncolytic virus may be insufficient to launch an anti-tumoural immune response. Therefore, most clinical trials on oncolytic-virus-based treatment are investigating combination approaches with chemotherapy, radiotherapy, CAR-T-cell and immune checkpoint blockade therapy to identify combination therapies that improve treatment outcomes.

Plant viruses and bacteriophages

Cowpea mosaic virus

In contrast to oncolytic viruses, non-mammalian viruses, such as plant viruses, do not infect mammalian cells; however, they can also be designed for intratumoural immunotherapy (Fig. 4b). In particular, non-cytolytic plant viruses, such as the cowpea mosaic virus (CPMV), can be repurposed for intratumoural immunotherapy. Plant viruses contain PAMPs that are recognized by PRRs and stimulate innate immune cells, thereby reprogramming

the TME to launch systemic and durable anti-tumour immunity upon intratumoural administration⁹. For example, in tumour mouse models and canine cancer patients¹²⁶, systemic efficacy (the abscopal effect) can be achieved by intratumourally administered CPMV (or VLPs thereof); here, both CPMV-injected and distant non-injected tumours shrink upon treatment with CPMV^{126,127} owing to durable CD8⁺ T-cell-mediated systemic anti-tumour responses that also prevent recurrence after re-challenge in mice⁹. Long-lasting protection has also been achieved in canine cancer patients (pets) with advanced mammary cancer, who received CPMV VLP intratumoural immunotherapy as neoadjuvant therapy prior to surgery¹²⁶.

CPMV interacts with the immune system in a multivalent manner, resulting in a cascade of events. Although CPMV is a plant virus, it resembles animal picornaviruses (Box 1) and is recognized by PRRs. Upon intratumoural delivery, the capsid proteins of CPMV interact with and stimulate TLR2 and TLR4; its positive strand single-stranded RNA (ssRNA) agonizes TLR7 and activates antiviral IFN signalling through the MyD88 pathway²³. Thus, intratumourally delivered CPMV polarizes the TME to an immune-activated phenotype, thereby transforming ‘cold’ (immune-suppressed) tumours with poor prognosis into ‘hot’ (immune-activated) tumours. Accordingly, CPMV treatment results in the infiltration and activation of innate immune cells, such as natural killer cells, anti-tumour neutrophils (N1), macrophages (which switch from an immune suppressive to a proinflammatory phenotype) and dendritic cells⁹. This immune reprogramming is generated in response to a suspected viral threat. The mammalian immune system does not discriminate between viruses from different kingdoms and reacts with antiviral responses if pathogen-recognizing receptors, such as TLRs, are activated, no matter the type of virus. Importantly, the mechanism of action of CPMV and other plant viruses is distinct from that of oncolytic viruses. Plant viruses are not pathogenic and do not lyse cancer cells directly, and so immune stimulation does not stem from viral replication or foreign protein synthesis. Although plant viruses, such as CPMV, act on innate immune cells, they also trigger adaptive and durable immunity (that is, activation of CD4⁺ and CD8⁺ effector and memory T cells). Therefore, plant virus immunotherapy interfaces with the immune system and restores normal function (that is, immunosurveillance), which kickstarts the cancer-immunity cycle. Moreover, anti-CPMV antibodies cannot neutralize the anti-tumour efficacy of CPMV (shown in an ovarian tumour model as well as canine cancer patients)^{128,129}. By contrast, anti-CPMV antibodies increase opsonization of CPMV and uptake by antigen-presenting cells, which increases, rather than reduces, efficacy, and is likely to be responsible for building the T-cell memory compartment¹²⁸.

Filamentous plant viruses and bacteriophages

Filamentous plant viruses are also being investigated for intratumoural immunotherapy. For example, the papaya mosaic virus (PapMV) of the *Alphaflexiviridae* family is a 530-nm flexuous virus that naturally infects papaya plants^{130,131}. PapMV VLPs were first developed as a vaccine platform against bacterial and viral infections^{132,133}, and are now also being explored for intratumoural immunotherapy. In PapMV VLPs, a packaged non-coding ssRNA functions as a TLR7 agonist^{22,134}, leading to the induction of type I IFN¹³³. In B16-OVA melanoma tumour mouse models, PapMV intratumoural immunotherapy substantially

reduced tumour burden, decreasing tumour proliferation markers and increasing major histocompatibility complex MHC-I surface expression on B16-OVA tumour cells²². In addition, the treatment led to an increase in chemokines (such as interferon- γ -induced protein 10, IP-10 and monocyte chemoattractant protein 1, MCP-1) and pro-inflammatory cytokine (such as IL-1 α and IL-5) concentrations in the TME, which can convert the TME immunotype from suppressed to activated²². The filamentous plant virus potato virus X (PVX) also shows anti-tumour efficacy in mouse models¹³⁵, triggering the upregulation of proinflammatory cytokines and chemokines, such as IL-1 α , IL1 β , IP-10 and MCP-1, thereby delaying tumour progression¹³⁵.

In addition to plant viruses, M13 bacteriophage (Fig. 5), a filamentous positive-sense ssDNA bacteria-infecting virus of the *Inoviridae* family, showed anti-tumour efficacy in mouse models^{8,136}. Within the TME, M13 stimulates a MyD88-dependent anti-tumour pathway¹³⁶, thereby promoting macrophage and neutrophil infiltration as well as the upregulation of antigen presentation and co-stimulatory receptors⁸. M13 is endocytosed and localizes to the endolysosome¹³⁷, where it functions as a TLR9 agonist based on its ssDNA cargo, highlighting the role of virus nucleic acid recognition in inducing tumour immunity¹³⁶.

Viruses and VLPs as delivery systems

The properties of both mammalian and plant viruses can be harnessed and engineered to achieve new functionality. However, in contrast to mammalian viruses that require controlled environmental conditions to function (that is, physiological pH and temperature), plant viruses and bacteriophages (and their derived VLPs) are more robust and can withstand a range of environmental conditions throughout their life cycle^{138,139}. Importantly, viruses can inherently serve as delivery vehicles, because they can encapsulate, protect and deliver nucleic acid cargo into their host cells for propagation. Therefore, viruses and VLPs can be repurposed as drug and gene carriers through genetic programming, bioconjugation or encapsulation¹⁴⁰, for example, for the delivery of genes encoding cytokines (for example, TVEC's genome encodes GM-CSF) and small-molecule drugs, such as STING and TLR agonists^{30,141–145}. Packaging of small-molecule agents, such as TLR agonists, into nanoparticles or VLPs overcomes their rapid leaching from tumours, protects them from enzymatic degradation and improves immune cell uptake, thereby boosting efficacy.

Vidutolimod as a Q β bacteriophage drug carrier

Also known as CMP-001, vidutolimod is a VLP derived from the Q β bacteriophage¹⁴⁶ of the *Leviviridae* family (Fig. 5). This bacteriophage has been engineered to carry unmethylated CpG-dense DNA (a TLR9 agonist). CpG molecules can activate immune cells and generate type I IFNs¹⁴⁷, but are limited by low tissue retention, rapid clearance and degradation by nucleases upon administration¹⁴⁸. In vidutolimod formulation, CpG is encapsulated in a Q β bacteriophage nanoparticle to circumvent these problems and enhance its multi-mechanistic action¹⁴³, which has been tested in several clinical trials ([NCT04698187](#), [NCT05445609](#) and [NCT04633278](#)). Upon intratumoural administration, vidutolimod remodels the TME by activating plasmacytoid dendritic cells to generate type I IFNs and other innate immune cells (such as natural killer cells¹⁴³), causing downstream

cross-talk with the adaptive immune system and priming of CD8⁺ T cells for anti-tumour activity¹⁴⁹.

Interestingly, in mouse models, the anti-tumour efficacy of vidutolimod depends on antibody-mediated immune-cell targeting to plasmacytoid dendritic cells and monocytes. Furthermore, the presence of antibodies in vitro increases immune-cell uptake of vidutolimod, and pre-immunization of mice before treatment also enhances its efficacy¹⁵⁰, because antibody opsonization of vidutolimod promotes immune-cell uptake through Fc-receptor engagement¹⁵⁰. Therefore, the clinical protocol requires induction of anti-drug antibodies through immunization against the Q β carrier VLP prior to treatment^{143,149,150}.

Other virus and VLP drug carriers

VLPs from CCMV can also be engineered to encapsulate or covalently display small-molecule agonists to target TLR7 (ref. 145). Agonists that target the STING pathway have been delivered by VLPs made of HIV-1 structural proteins combined with the envelope glycoprotein from vesicular stomatitis virus (VSV)¹⁵¹. Another VLP drug carrier example is Ad5D24–CpG, which is an oncolytic adenovirus with unmethylated CpG DNA synthetically engineered into its genome to enable delivery and targeting of TLR9 (ref. 152).

Outlook

Various virus nanotechnologies have been tested for intratumoural immunotherapy, but only TVEC has been approved for clinical use by the FDA thus far. The efficacy of virus-nanotechnology-based intratumoural immunotherapy might be limited by the presence of pre-existing neutralizing antibodies. In addition, achieving the translation of local to systemic efficacy, that is, the abscopal effect, remains challenging. Intratumoural immunotherapy can reverse immunosuppression within the injected tumour; however, immune-cell recruitment to distant non-injected tumours remains difficult to achieve, thereby limiting treatment success. Although the abscopal effect has been reported in patients^{10,123,124}, it is considered a rare and unpredictable event.

In addition, although targeting nucleic-acid-recognition receptors (such as TLRs and the STING pathway) can promote the anti-tumour immunity of virus-based therapies by launching antineoplastic type I IFN responses, it also triggers antiviral programs that may reduce the ability of oncolytic viruses to replicate and lyse tumour cells. Indeed, retinoic-acid-inducible gene I (RIG-I) detection of viral RNA can negatively regulate oncolytic efficacy, and STING signalling activated through viral double-stranded DNA recognition can interfere with the efficacy of oncolytic viruses^{153,154}. Therefore, the right balance between an anti-tumour and an antiviral response must be considered when developing virus-based therapies — a balance that is an inherent characteristic of the immune system and the cancer-immunity cycle.

Furthermore, intratumour mutational heterogeneity and related T-cell priming in mismatch-repair-related tumour models may be a limiting or promoting factor in immunotherapy^{155,156}. That is, levels of mutational burden in tumours as well as the diversity of mutations can have a role in the immunotherapy response, and may have to

be considered in the design of virus-based immunotherapy. However, the mechanism of virus nanotechnology is considered to be tumour-agnostic: the intention is to overcome these limitations by releasing tumour antigens into the TME through oncolysis (either promoted directly by an oncolytic virus or indirectly through recruitment of natural killer cells or neutrophils by a VLP or virus) and by serving as an adjuvant for the immunological processing of the released antigens. Therefore, a particular mutation of an antigen may not necessarily interfere with the mechanisms of virus platforms. Mutations may even create a favourable environment for oncolytic viral activity⁸⁹. However, specialized infrastructure and training may be required to produce replication-competent oncolytic viruses¹⁵⁷. Similarly, although they are safe, the manufacture of plant viruses and VLPs may involve specialized plant molecular farming platforms or multi-step assembly approaches for packaging therapeutic cargo (Box 2).

Various combination strategies are currently tested in clinical trials to improve immunotherapy outcomes. In particular, immune checkpoint therapy has shown clinical responses¹⁵⁸, thereby driving its integration into first- and second-line therapies. However, only a minority of patients respond to immune checkpoint therapy, largely because the immunosuppressive TME contains physical and/or chemical barriers to effective T-cell anti-tumour immunity¹⁵⁸. Preclinical studies have shown that intratumoural immunotherapy with VLPs encapsulating small-molecule agonists, plant viruses or oncolytic viruses synergize with immune checkpoint therapy (for example, treatment with anti-PD-1 antibodies) by increasing the expression of checkpoint markers within the TME and by expanding the pool of tumour-specific CD8⁺ effector T cells^{159–162}. Of note, vidutolimod is undergoing clinical testing as both a solo therapy and with an immune checkpoint therapy combination arm^{163–165}. Moreover, for virus and VLP drug carriers that are not directly cytotoxic, combination with treatment regimens that lyse tumours and release tumour antigens (chemotherapy, cryoablation, photothermal therapy and radiation) hold promise for holistic immunotherapy. Combination approaches could be implemented with virus-based intratumoural immunotherapy as an adjuvant or neoadjuvant therapy. For example, neoadjuvant treatment with TVEC prior to surgery improves recurrence-free survival in human patients¹⁶⁶.

Accessibility is a key requirement for intratumoural therapy, which may be challenging to achieve for disseminated peritoneal cancers (ovarian and colon cancers), metastatic disease or haematological cancers³⁰. However, interventional radiology and image-guided procedures may improve accessibility in such cancers. In addition, delivery techniques (for example, multiside hole needles) and slow-release devices^{167,168} that can overcome barriers, such as high interstitial fluid pressure in tumours limiting drug penetration³⁷, may be applied. Delivery devices and slow-release depots may also be designed to avoid repeated intratumoural dosing, which adds to treatment costs and may cause discomfort, thereby affecting patient compliance^{38,169}. Given their robust nature, plant viruses and bacteriophages are particularly well suited to be integrated into medical devices³⁹.

A better understanding of the underlying mechanisms of virus–host cell interactions, the clinical application of cancer immunotherapy and the safety and affordability of intratumoural immunotherapy as well as its rapid systemic anti-tumour response (weeks

between diagnosis and surgery) suggest that virus-nanotechnologybased intratumoural immunotherapy may well become integrated into standard-of-care cancer treatments. However, how best to generate an abscopal effect and how to technically combine various immunotherapies remains to be identified.

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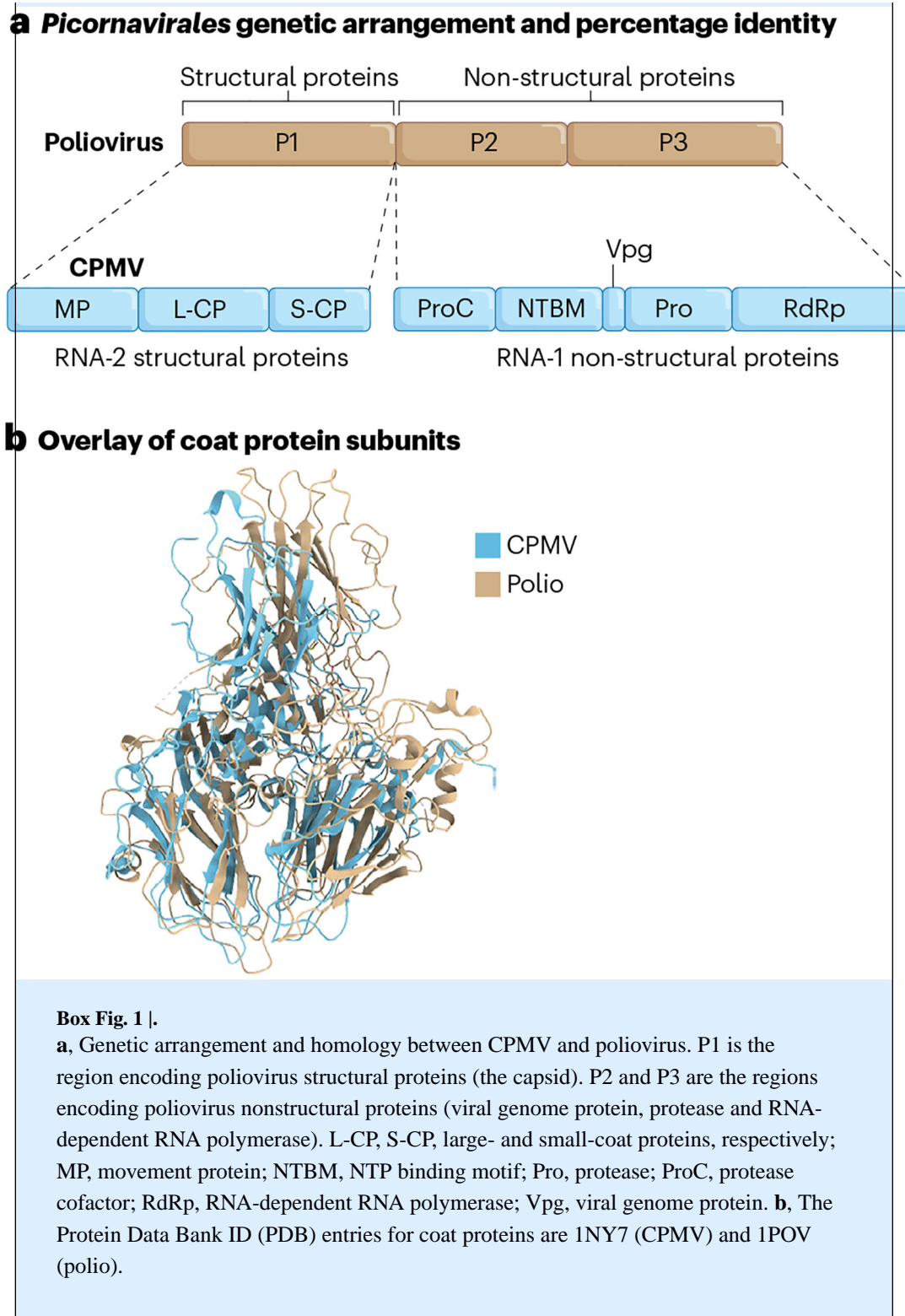
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Box 1 |**Virus taxonomy informing nanomedicine design: a case for *Picornavirales***

Several *Picornavirales*-based intratumoural immunotherapy strategies have been developed, including PVSRIPO, CAVATAK and GD7-KS1. These viruses contain picorna-RNA (a small RNA genome) and are positive-sense RNA viruses with an icosahedral morphology¹⁸⁶, with a diverse range of hosts, including insects, vertebrates and plants. However, their structural morphology and genetic arrangement remain homologous across species¹⁸⁷ (see Box 1 figure panels **a** and **b**). The structural recognition of virus motifs by the host-immune system contributes to their potent anti-tumour immunity. For example, in addition to the oncolytic activity of PVSRIPO, its RNAs are recognized by the cytoplasmic pattern recognition receptors (PRRs) mitochondrial antiviral signalling protein (MAVS), melanoma differentiation associated gene 5 (MDA5) and retinoic acid-inducible gene I (RIG-I), resulting in the generation of type I interferon (IFN)^{188,189}. CPMV RNAs are recognized by Toll-like receptor (TLR)-7, which activates myeloid differentiation primary response 88 (MyD88) signalling to generate type I IFN²³. Different types of PRR recognize the different viruses, probably owing to differences in intracellular processing; upon cell entry, PVSRIPO uncoats and introduces its RNA into the cytoplasm for translation¹⁸⁸, whereas CPMV localizes in the endolysosome for an extended period, where its RNAs agonize TLR7 (ref. 23).

Cowpea mosaic virus (CPMV) is from the *Secoviridae* family (within the *Picornavirales* order) that naturally infects beans and legumes¹⁹⁰. Other plant viruses that are not in the picorna family, such as cowpea chlorotic mottle virus (CCMV), sesbania mosaic virus (SeMV) and physalis mottle virus (PhMV), show no efficacy as intratumoural immunotherapy agents¹⁹¹. However, cowpea severe mosaic virus (CPSMV) and tobacco ringspot virus (TRSV) — also members of the plant picornaviruses — show potency against tumours in mouse models, albeit with reduced efficacy, reflected in reduced type I IFNs and TLR7 stimulation, compared to CPMV¹⁹². The potency of CPMV may be related to the conserved structure and genetic organization between plant and animal picornaviruses, such as the polio virus (see Box 1 figure panels **a** and **b**)^{193,194}. In addition, antigens are shared between plant and mammalian picornaviruses¹⁹⁵, which may suggest a common ancestor.



Box 2 |**Translational considerations for plant viruses and virus-like particles**

The clinical translation of plant viruses and plant virus-like particles (VLPs) faces several challenges. In particular, plants are typically not used for biomanufacturing¹⁹⁶, knowledge about manufacturing of biologics in plants is limited (for example, compared to Chinese hamster ovary cells, which are often used for biomanufacturing), and only a few contract development and manufacturing organizations have been established to facilitate process development and current good manufacturing practice (cGMP) manufacturing. In addition, many reagents and assays for the production and quality control of plant-virus-based products differ from those used for mammalian systems and are thus not readily commercially available.

Plant-based biomanufacturing also requires custom-designed and contained growth facilities, whereas upstream production equipment, such as bioreactors, is available off the shelf. Typically, host plants are manually infected with viral stocks or transfected by recombinant transfer DNAs, delivered by *Agrobacterium tumefaciens*, which can be difficult to scale up. To obtain the clarified extract, each combination of plant host and virus requires process optimization¹⁹⁷, and laboratory processes need to be adapted to robust, high-yield and scalable industrial unit operations. In addition, although the large size difference between plant viruses and plant host proteins (for example, plant viruses typically have a size of 3,000 kDa, which is ten times bigger than the plant host cell protein RuBisCo, of ~500 kDa) is an advantage for ultrafiltration, the high mass transport and size exclusion are disadvantages for column chromatography. Importantly, scalable systems are being developed for plant-virus-based vaccine production; for example, the Coalition for Epidemic Preparedness Innovations (CEPI) has funded LenioBio's plant-cell-lysate-based technology for vaccine production¹⁹⁸.

For the scale-up translation of plant viruses and VLPs, regulatory guidelines for cGMP-compliant biomanufacturing can be adapted from existing plant-based biologics¹⁹⁹. In addition, turnkey vertical farming solutions with low footprint, high yield, automation and energy efficiency are being marketed. Such scalable systems cover all scale requirements, from initial clinical development to marketing. Automation of the manual infection process can be achieved with the aid of robotics, camera systems and artificial intelligence. Large-scale extraction and clarification are routinely done in the food industry; however, developing suitable down-scale models remains difficult. Filtration technology has high scalability and is available off the shelf, and further downstream processing could be designed as in approaches used for non-enveloped oncolytic viruses. Cost models for large-scale plant-based cGMP manufacturing facilities have demonstrated economic viability for several products that have higher dosage than those required for viral nanotechnology for intratumoural immunotherapy^{200–202}.

Key points

- Viruses are immunomodulatory biologics that can be repurposed for intratumoural immunotherapy to kickstart the cancer immunity cycle.
- Mammalian viruses, non-mammalian viruses and virus-like particles can be engineered to trigger immune responses or deliver therapeutic cargo for immunotherapy.
- Virus-associated pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) recognition (through replication, nucleic acid payload, protein expression or structure) can induce type I interferon (IFN) responses and promote anti-tumour immunity.
- Intratumoural immunotherapy using virus-based nanomaterials and gene-delivery vectors benefit from low costs and dose requirements as well as minimal side-effects and systemic toxicity.

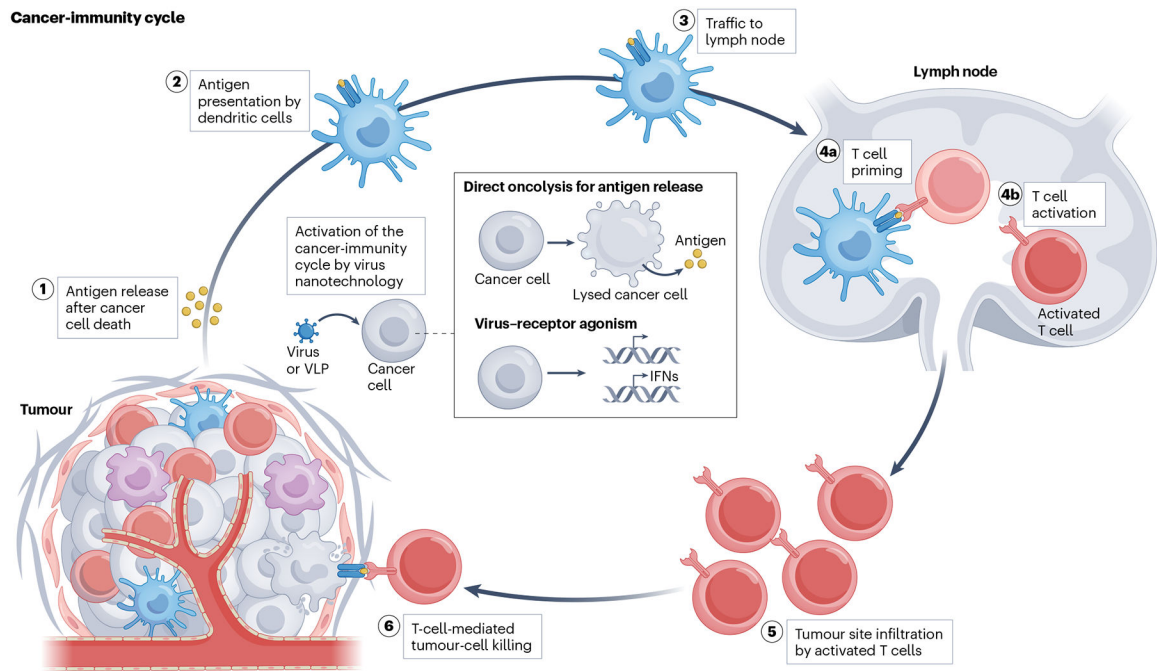


Fig. 1 |. Cancer-immunity cycle.

Antigens are released from dead or dying cancer cells. These antigens are then presented by dendritic cells and trafficked to lymph nodes, where T cells are primed and activated. Activated T cells infiltrate tumour sites to induce more cancer-cell death. The cancer-immunity cycle can be activated by oncolytic viruses that infect cancer cells to trigger their oncolysis and subsequent antigen release. Alternatively, viruses and virus-like particles (VLPs) can be designed to bind to specific receptors on cancer cells, promoting stimulation of the immune system by stimulating type I interferon (IFN) signalling.

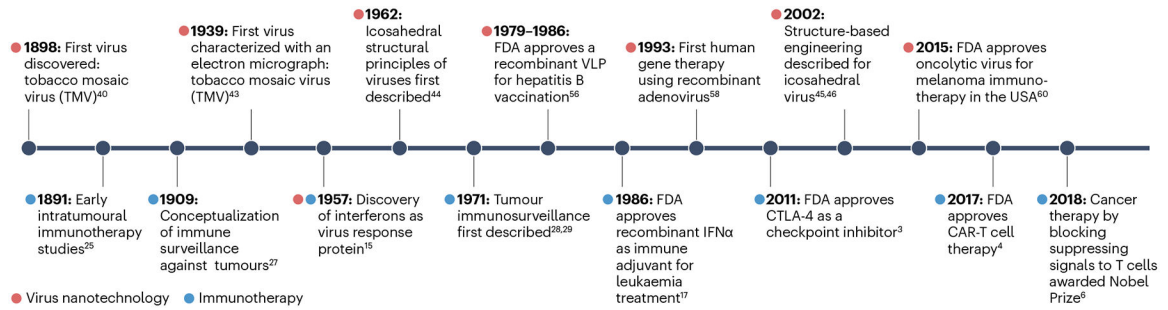


Fig. 2 |. Milestones of virus nanotechnology and immunotherapy.

CAR-T cell, chimeric antigen receptor T cell; CTLA-4; cytotoxic T-lymphocyte-associated antigen 4. Data are taken from refs. 3,4,6,15,17,25,27–29,40,43–46,56,58,60.

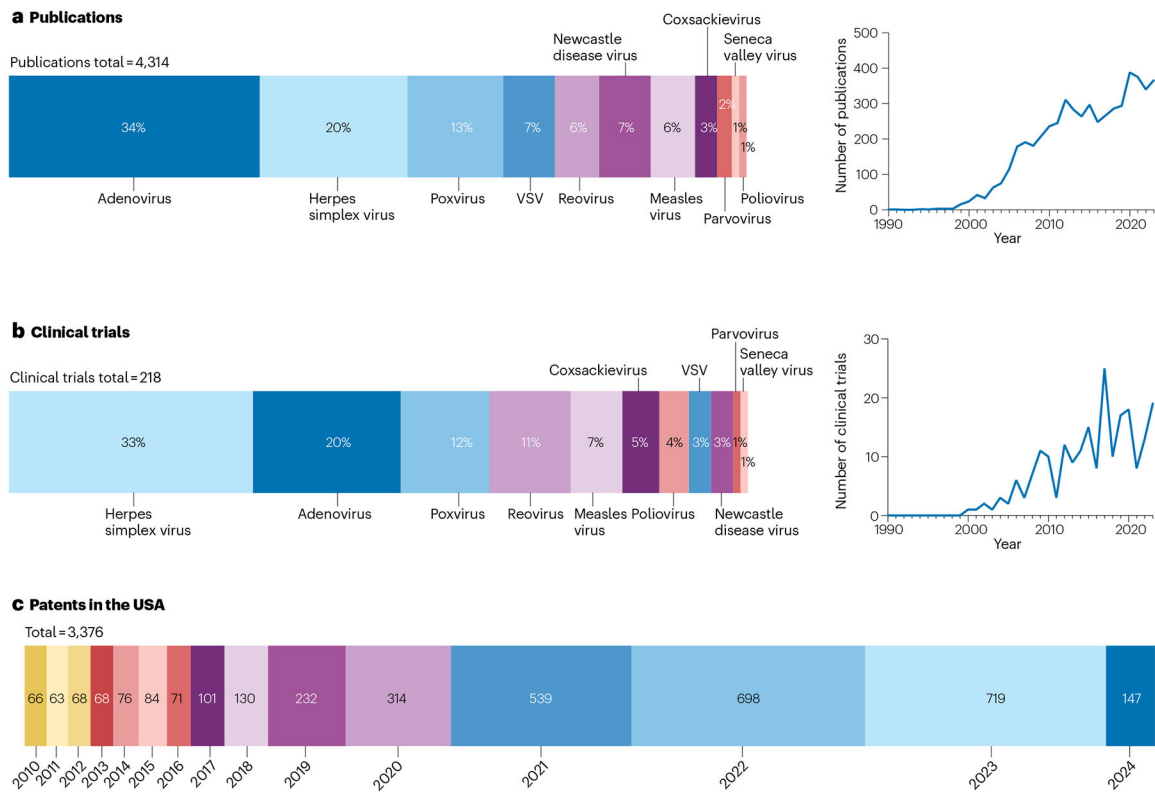


Fig. 3 |. Research and investment in oncolytic viruses.

a, Peer-reviewed publications in 1990–2024 involving different types of oncolytic virus.

Search terms in PubMed: [oncolytic virus] AND [cancer] AND [virus type name]. **b**,

Clinical trials using oncolytic viruses in 1990–2024. Oncolytic viruses undergoing clinical

trials were extracted from the [Clinicaltrials.gov](https://clinicaltrials.gov) database using the following keywords in

titles and abstracts: oncolytic virus, adenovirus, poxvirus, vaccinia, coxsackievirus, herpes

simplex virus (HSV), measles virus, Newcastle disease virus, parvovirus, reovirus, Seneca

Valley virus, vesicular stomatitis virus (VSV), poliovirus and cancer. **c**, Patents filed for

oncolytic-virus platforms from 2010 to 2024 in the USA. Search terms in Google patents

include [oncolytic virus] AND [cancer] + [country = USA].

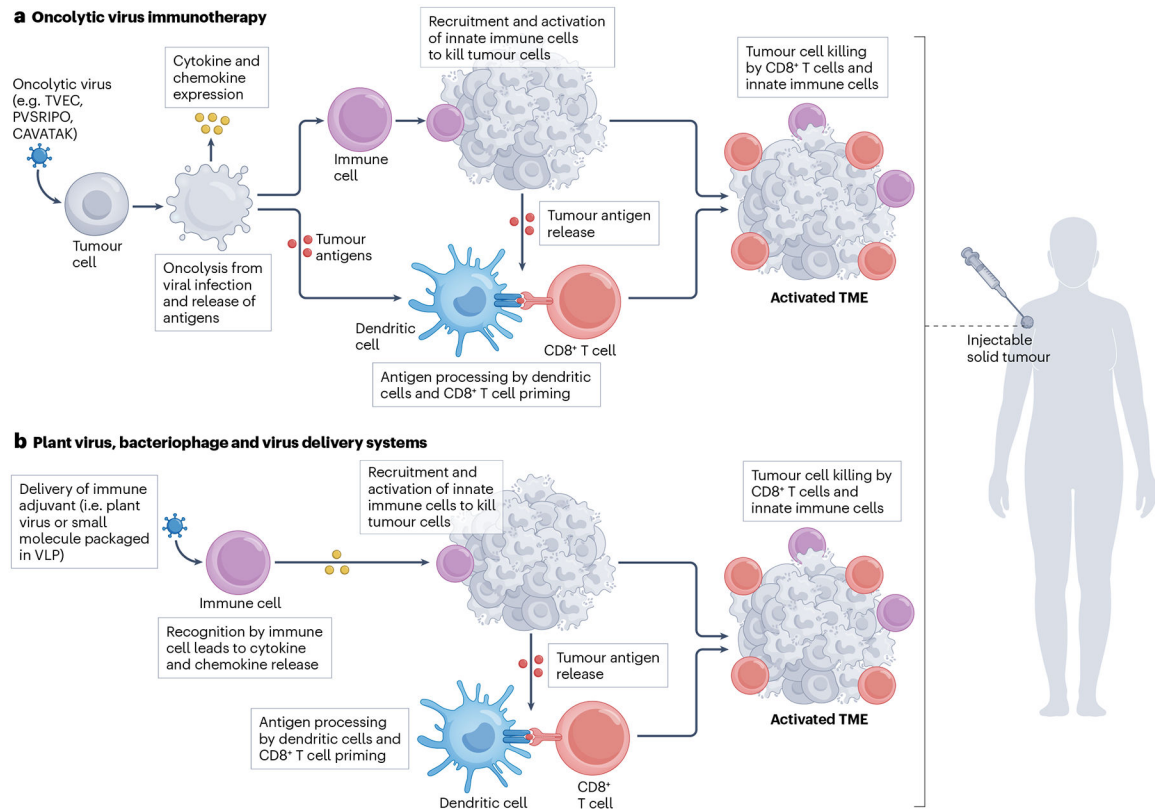


Fig. 4 |. Mechanism of action of virus-based intratumoural immunotherapy.

a, Oncolytic viruses selectively infect and lyse tumour cells to release antigens. Antigens are used by dendritic cells to prime CD8⁺ T cells, which induce tumour killing. **b**, Plant viruses and virus-like particles (VLPs) are recognized by the immune system, which, in response, launches an activation programme. Recognized antigens prime CD8⁺ T cells for tumour killing. TME, tumour microenvironment; TVEC, herpes simplex virus 1; PVSRIPO, poliovirus; CAVATAK, coxsackievirus.

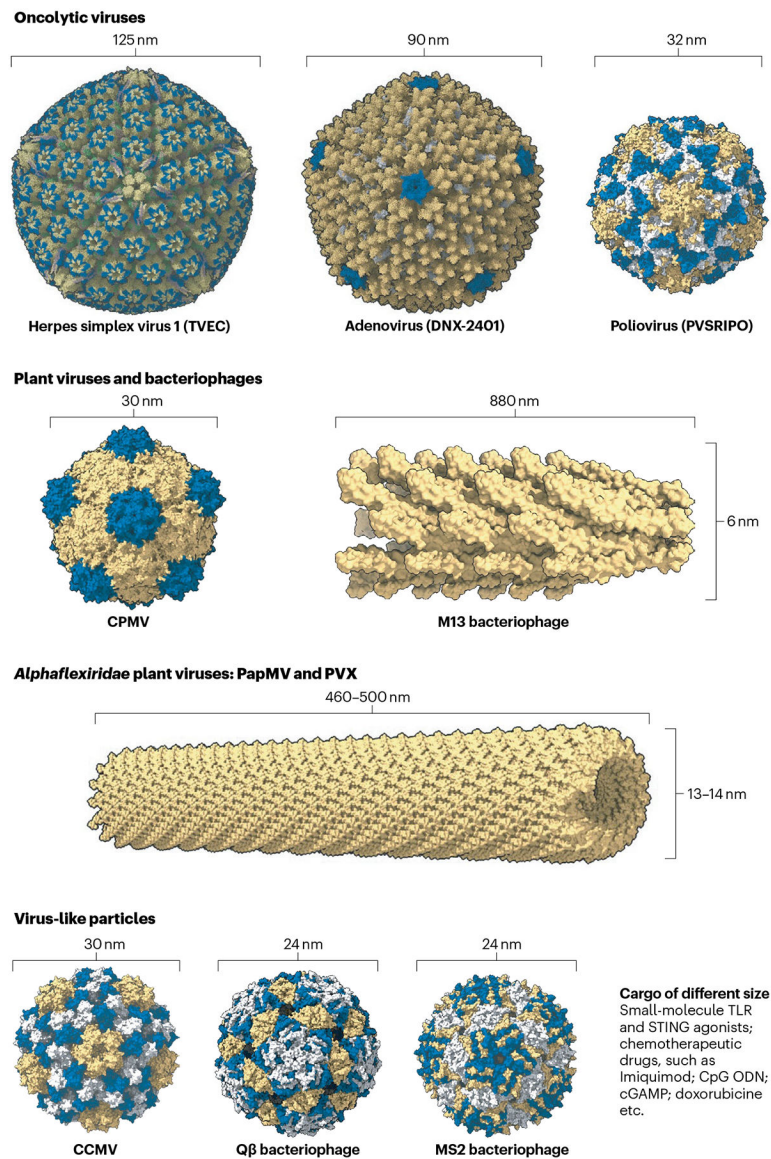


Fig. 5 |. Structure and scale of viruses and virus-like particles used for intratumoural immunotherapy.

Virus and virus-like particle (VLP) structures are diverse. This allows them to be engineered for intratumoural immunotherapy. Mammalian viruses include the herpes simplex virus 1 (TVEC; Protein Data Bank ID (PDB): 6CGR), adenovirus (DNX-2401; PDB: 6CGV) and poliovirus (PVSRIPO; PDB: 1POV). TVEC is currently approved for melanoma, and DNX-2401 and PVSRIPO are currently being tested in clinical trials. Cowpea mosaic virus (CPMV; PDB: 1NY7), M13 bacteriophage (PDB: 2MJZ), the *Alphaflexiridae* plant viruses papaya mosaic virus (PapMV) and potato virus X (PVX; PDB: 4DOX), cowpea chlorotic mottle virus (CCMV; PDB: 1ZA7), Q β bacteriophage (PDB: 1QBE) and MS2 bacteriophage (PDB: 2MS2) are currently in the preclinical development pipeline. Viruses and VLPs can deliver Toll-like receptors (TLRs) and stimulator of interferon gene (STING) agonists.

Table 1 |

Selected completed clinical trials of oncolytic viruses

Virus type	Drug	Cancer type	Clinical trial number	Phase	Refs.
Adenovirus	CG0070	Bladder cancer	NCT02365818	Phase 2, single group, open label	170
	DNX-2401	Glioblastoma, gliosarcoma	NCT02798406	Phase 2, single group, open label	171
		Brainstem glioma	NCT03178032	Phase 1, single group, open label	172
Picornavirus	NSC-CRA4-S-p7	Glioma	NCT03072134	Phase 1, open label	173
	PVSRIP0	Melanoma	NCT03712358	Phase 1, non-randomized, open label	174
	SVV-001	Solid tumours with neuroendocrine features	NCT00314925	Phase 1, non-randomized, open label	175
Herpes simplex virus	CVA21 (CAVATAK)	Malignant melanoma	NCT01227551	Phase 2, single group, open label	123
		Uveal melanoma	NCT03408587	Phase 1b, randomized, open label	176
	TVEC	Melanoma	NCT00289016	Phase 2, single group, open label	177
Parvovirus		Melanoma	NCT00769704	Phase 3, randomized, open label	10,178
		Melanoma	NCT01740297	Phase 1/2, randomized, open label	179
	HSV1716	Non-central nervous system solid tumours	NCT00931931	Phase 1, single group, open label	180,181
Poxvirus	H-1PV	Glioblastoma	NCT01301430	Phase 1/2a, single group, open label	182
	JX-594	Melanoma	NCT00429312	Phase 1/2, single group, open label	183
		Hepatocellular carcinoma	NCT00554372	Phase 2a, randomized, open label	184
	GL-ONC1 (Olvi-Vec)	Ovarian cancer, peritoneal carcinomatosis	NCT02759588	Phase 1b/2, single group, open label	185

Data extracted from the [Clinicaltrials.gov](https://clinicaltrials.gov) database using the following keywords: oncolytic virus, adenovirus, poxvirus, coxsackievirus, herpes simplex virus, parvovirus, reovirus, Seneca Valley virus, vesicular stomatitis virus, poliovirus and cancer.