Achieving systemic delivery of oncolytic viruses

Claudia Hill & Robert Carlisle

To cite this article: Claudia Hill & Robert Carlisle (2019) Achieving systemic delivery of oncolytic viruses, Expert Opinion on Drug Delivery, 16:6, 607-620, DOI: 10.1080/17425247.2019.1617269

To link to this article: https://doi.org/10.1080/17425247.2019.1617269

© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

Published online: 30 May 2019.

Submit your article to this journal

Article views: 1245

View related articles

View Crossmark data
Achieving systemic delivery of oncolytic viruses
Claudia Hill and Robert Carlisle
Institute of Biomedical Engineering, University of Oxford, Oxford, UK

ABSTRACT

Introduction: Oncolytic virotherapy is a selective and powerful tool for cancer treatment. Studies proving the ability of oncolytic viruses (OVs) to target and rapidly kill cancer cells have led to approval of H101 and Imlygic®. Both these OVs are restricted to intratumoral administration into cancer lesions. Despite promising preclinical results, systemic delivery of OV has shown limited success in patients due to a knockdown in infectivity, as a result of rapid immune-mediated neutralization, and poor penetration into tumors.

Areas covered: This review catalogs the techniques used to enhance OV delivery. Firstly, insights from clinical trials of OV provide evidence of the need for enhanced delivery strategies. Secondly, the techniques applied to overcome the challenges highlighted by clinical trial data (i.e. suboptimal pharmacokinetics, antiviral immune responses, and poor penetration into solid tumors) are reviewed.

Expert opinion: For OV to gain traction and convert potential into value, researchers focussed on showing clinical and commercial viability following intratumoral injection. For the technology to mature and become applicable across a wider range of patients/cancer indications, amenability to systemic delivery is required. This may be achieved using strategies that modulate the OV by genetic or chemical means and/or that alter the physiology of target tumors.

1. Introduction

Traditional cancer treatments are not optimally effective; their mechanism of action commonly relies on specificity solely derived from the enhanced division rate of cancerous cells. Consequently, any rapidly dividing cells are targeted, which results in dose-limiting side effects that can exacerbate the suffering caused by the disease itself. Examples of the healthy cells in the body that share a high division rate include hair cells, bone marrow cells, and cells in the gut [1] and so these are also attacked and killed during chemotherapy treatments, leading to hair loss, vomiting, and myelosuppression. Fortunately, treatment modalities for cancer have begun to move far beyond these traditional approaches as our understanding of cancer has improved. While there have been several advances in the last 10 years that have seen increased survival rates in some common solid tumors, the most significant change has come from understanding the genetic and molecular basis of cancer [2]. This knowledge has led to the development of new types of therapy including biologics and gene therapies, which have allowed the biomedical community to start to treat cancer in a more targeted way. This research has led to the development of more powerful and selective approaches which do not instigate the same off-target toxicity profiles observed with conventional small drug chemotherapies. One such novel biologic gene-based therapy is known as ‘oncolytic virotherapy’ whereby viruses with a natural or engineered ability to infect and kill cancer cells are utilized.

(1) Oncolytic Virotherapy: Benefits and Current Restrictions

References to the use of viruses to treat cancer patients, dating back to the early 1900s, report that cancer regression prevailed when patients became the subject of naturally occurring viral infections. This lead to trials in which bodily fluids containing human or animal viruses were administered to cancer patients. The outcomes of these studies were largely negative due to viral neutralization by the host immune system which prevented cancer cell infection and therefore tumor regression from occurring. In certain immunosuppressed patients, tumor regression was reported, however due to the lack of specificity of the viruses used, a number of patients died as a results of viral infection of normal tissue [3]. Oncolytic virotherapy has since advanced as a result of a greater understanding of virology, an ability to scale-manufacture higher quality oncolytic virus (OV) batches, and less reliance on the natural tumor tropism and more genetic modification to achieve improved selectivity of viruses for cancer cells. In 1989, it was first reported that a herpes simplex virus type 1 (HSV-1) with an inactivated thymidine kinase (TK) gene led to tumor control without associated encephalitis in mice [4]. This study was the inspiration for many more investigations into oncolytic virotherapy [5,6].

Viruses are commonly used in medical treatments as vectors for gene delivery and, more recently, as therapeutics for targeted cancer treatment either via cell destruction or by stimulation of an immune response against the cancer cells or a combination of both these events [2,5–7]. Four key
With so many advantages it is not surprising that in recent years there has been a surge in the development and testing of OV for cancer treatment. Results from a plethora of preclinical and clinical studies using HSV [14–18], vaccinia virus (VV) [19–26], and adenovirus (Ad) [27–36] for cancer treatment, have provided exciting evidence which has led to strengthened clinical and commercial interest in OV [37].

There are now two OV which have been approved for clinical use: H101 and Talimogene Laherparevoc (Imlygic®). In 2005 the China Food and Drug Administration (CFDA) approved H101, an E1B-deleted serotype 5 Ad, for intratumoral (IT) administration in combination with chemotherapy in the treatment of head and neck cancer [38]. Imlygic®, was first described in 2003 [39], it is a HSV type 1 (HSV-1) derived by functional deletion of ICP34.5 and ICP47 and insertion of the coding sequence for human granulocyte macrophage colony-stimulating factor (GM-CSF). In 2015, Imlygic® was the first OV to gain FDA approval for IT administration in patients with inoperable malignant melanoma [40,41]. When administered every 2 weeks IT into accessible lesions, Imlygic® produced systemic responses in 16% of patients and longer overall survival of 23.3 months in the Imlygic® arm compared to 18.9 months in the control arm [42].

CAVATAK, a bioselected genetically unmodified oncolytic Cocksackievirus A21 (CVA21) [43], has also been the subject of Phase I and II clinical trials. A Phase II (NCT01227551) study looking at the use of CAVATAK in patients with late-stage melanoma showed that IT delivery of CVA21 was safe (no Grade 3 or 4 product-related adverse effects) and resulted in an overall response rate of 28.1% [44].

Two further Phase Ib and Phase I clinical investigations into the IT administration of CVA21 in combination therapy with impililumab (NCT02307149) and pembrolizumab (NCT02565992) in patients with advanced melanoma are still ongoing.

ReoLysin®, a serotype 3 oncolytic reovirus, has also shown safety and activity in Phase I and II studies against breast cancer (NCT01656538), multiple myeloma (NCT02514382), and nonsmall cell lung cancer (NCT01708993). The first Phase II randomized breast cancer study (NCT01656538) assessed the intravenous administration of ReoLysin in combination with a chemotherapeutic (paclitaxel) [45]. The study showed that there was no significant increase in progression-free survival (PFS) or response rate (RR) between those treated with paclitaxel alone and those treatment with a combination of ReoLysin and paclitaxel. Treatment with the combination of ReoLysin and paclitaxel did however provide extended overall survival (from 10.4 months to 17.4 months). Data determining the number of viral particles in the tumors were not reported and so it is not known whether poor delivery was a factor in the failure to improve PFS or RR [45]. Notably, it has also been reported that higher levels of PD-L1 was expressed in tumors resected from patients with glioblastoma multiforme who had been treated intravenously with ReoLysin [46].

Oncolytic VV has also shown promise and has been tested in hundreds of cancer patients in late-stage clinical trials to date. These trials have determined how oncolytic VV can be administered safely and efficiently in multiple types of cancer. Pexa-Vec, a Wyeth strain VV with TK deleted and encoding GM-CSF, was first investigated in clinical trials in 1999 by

benefits of virotherapy [8] can be summarized as selectivity, replication, arming potential, and provision of extra/alternative death mechanisms.Viruses can be made selective in at least three ways: (a) modification of their surface so they bind target cells, (b) placement of important genes downstream of cancer specific promoters, and (c) deletion of genes which are required to allow replication in normal cells but have limited effect on replication in cancer cells [4,9,10]. An OV can self-amplify intratumorally, hence increasing its dose in situ, and so even a low-dose virus has the potential to generate a powerful response provided it is well distributed. Viruses can also be armed to produce a huge range of proteins from within the tumor which can increase the anticancer effect [11]. Finally, viruses provide mechanisms of death (e.g. necrosis [12]) which are not reliant on the activity of mediators of apoptosis such as p53 or Rb. Mutations to p53 and Rb are very commonly part of the process of cancer development as they prevent apoptotic death mechanisms, which consequently leads to loss of growth regulation. Many small molecule chemotherapy drugs rely on apoptotic death mechanisms to achieve their effect, i.e. they rely on the function of a process to treat a disease which is often defined by the loss of that process [13].
In 2005 in a Phase I study, the maximum tolerated dose (MTD) of IT injection in patients with metastatic or primary liver cancer was determined to be $1 \times 10^9$ pfu [21]. Clinical studies investigating the safety and efficacy of intravenous (IV) administration of Pexa-Vec were first reported in 2011 [26]. The trial administered escalating doses of Pexa-Vec in 23 patients with treatment-refractory solid tumors with the primary aim of determining the safety and MTD. No dose limiting toxicities (DLT) were reported at the highest dose administered, $2 \times 10^9$ pfu which was concluded to be the maximum feasible dose (MFD). Of significance, it was determined during this trial that the viremic threshold for tumor infectivity after IV injection was $1 \times 10^9$ pfu, below which there was no evidence of Pexa-Vec infection. The outcomes of both the IT and IV Phase I clinical trials [21,26] provided evidence for further late-stage clinical trials with Pexa-Vec. A randomized Phase II trial [47] investigating IT-administered Pexa-Vec in 30 patients with liver cancer was conducted to compare the outcomes of a high- and low-dose arm. The higher dose arm had a median survival of 14.1 months compared with 6.7 months at the lower dose. This was the first randomized clinical trial of an OV which demonstrated improvement in survival duration linked to the administered dosage. Another Phase II trial [48] in patients with hepatocellular carcinoma (HCC) reported the safety and efficacy of a combined treatment of Pexa-Vec IV and repeated IT injection followed by Sorafenib. After treatment with Pexa-Vec alone 62% of patients had disease control (as determined by RECIST criteria) and after 6–12 weeks 59% of patients had disease control. Both these clinical studies presented the clear therapeutic benefit of Pexa-Vec in patients with HCC. Further investigations into IV administration of oncolytic VV, the Phase Ila (NCT01636284) ‘FLASH’ and large Phase Iib (NCT01387555) ‘TRAVERSE’ studies, have now been completed. The results of these trials are yet to be published however, in the TRAVERSE trial, it was noted that patients with advanced liver cancer did not reach their primary survival endpoint even in the high-dose cohort [49]. An ongoing Phase III trial (NCT02562755) ‘PHOCUS’ is investigating the benefits of combined IT Pexa-Vec + oral Sorafenib compared to oral Sorafenib alone. The implication of the results of these later stage clinical trials could lead to high impact multimodal therapy for patients with HCC. There are two other oncolytic VV derivatives of note in early-stage clinical investigation GL-ONC1 (an attenuated version of the Lister strain with the deletion of F14.5L, A56R, and J2R) and the vVDD-CDSR (a Western Reserve derivative with TK and VGF deletions).

Crucially, out of the 24 studies reported investigating oncolytic VV clinically, only eight used IV administration. As improved survival has only been shown with IT administration, this may be the reason that most ongoing or recruiting trials are using IT administration (NCT02562755, NCT02977156, and NCT03071094). Hence, while IT administration of oncolytic VV has shown a safe profile with promising antitumor response, these trials have also indicated that effective dissemination throughout the body to treat metastatic sites of disease is still an area which needs improvement. At the MTD, IV administration of Pexa-Vec showed infection of tumors but the number of infectious VV virons that were able to reach tumor sites was limited and not enough to produce significant tumor regression as a monotherapy. It is notable that both H101 and Imlygic® are administered IT.

Hence, there are still important challenges to overcome before the use of OVs as therapeutic agents in cancer treatment becomes a standard clinical approach for treatment of metastatic disease. This restriction to IT administration is the result of several challenges faced by OV. Firstly, there is the challenge of delivering the virus via the bloodstream so that all the distant sites of cancer deposition can potentially be reached. When injected into the body, most viruses are recognized as xenogens and are attacked and cleared from the body before they are able to reach the desired location(s). This immune-mediated clearance dramatically impedes the efficiency of OV [26,50,51]. If the virus does manage to reach the tumor site, it then needs to have maintained a sufficient level of activity to be able to infect and kill cancer cells. This leads to the second challenge; the virus needs to be stable in the blood and retain a high level of infectivity. Thirdly, dense stromal tissue and poor lymphatic drainage within tumors make them high-pressure environments comprised of a dense disorganized collection of cells [5]. This means solid tumors often have no convective flow passing through them, making it difficult for particulates such as viruses to penetrate into and distribute throughout the whole tumor. Therefore, mechanisms to help the virus continue to infect, propagate, and spread until the entire tumor has been eradicated may be required [52].

2. Enhancing OV efficacy by overcoming delivery limitations

OVs have shown efficacy when delivered by direct IT injection both locally through direct tumor cell lysis and systemically by stimulating an antitumor immune response [42,53] (detailed in Section 1). However, in order for OV to become an established tool in pan-cancer treatment, able to optimally infect primary and metastatic tumors, they will need to be delivered via IV injection [7,52]. Indeed, IV injection may enhance the generation of antitumor immune responses compared to intralesional delivery [54] and provides enhanced benefit in cases where metastatic variants have lost or changed their neoepitope landscape which results in previous neoepitope specific treatments becoming nonefficacious. Moreover, IV administration is the standard, and more standardizable, means of therapeutic delivery worldwide and cancer treatment centers are set up with IV infusion equipment accordingly. Therefore, whilst IT delivery will continue to provide a viable option for many patients, achieving systematic bioavailability in local and disseminated cancer will be critical to the expansion of the utility and efficacy of OV.

2.1. Pharmacokinetics and antiviral neutralization

Providing better circulation kinetics following IV injection will enable more effective treatment of metastatic disease and hence widen the number of patients that can be treated with these powerful and selective agents. It has been shown in preclinical models that the bloodstream stability and
tumor accumulation of OV can be improved using modification or shielding techniques including those which rely on genetic and/or chemical engineering [8].

2.1.1. Directed evolution and genetic modification

Directed evolution, a technique which was the subject of the 2018 chemistry Nobel Prize, has been used as an alternative approach to producing highly selective and efficacious OV. It was first used in the OV field by Kuhn et al. for the creation of an oncolytic Ad that was not based on Adenovirus, namely ColoAd1 [55]. Here the authors pooled a variety of serotypes of Ad, which showed lower sero-prevalence in humans, and passaged them under conditions which closely mirrored those of the human cancer microenvironment. The viruses which thrived in these selective culturing environments were tested and those which showed increased potency and enhanced tumor tropism were selected. In the case of ColoAd1, the specific conditions were those of the colon cancer cell line, HT29. Tests were performed to determine ColoAd1’s activity in whole human blood and showed that compared with the Ad5-based OV ONYX-015, the infection inhibition in 20% (v/v) human serum was just 10-fold as opposed to 1000-fold. Both in vitro and in vivo investigations of ColoAd1 showed a 2–3 logs higher potency and a 3–4 logs increase in therapeutic window, when compared with Ad5 vectors [55]. The success reported with ColoAd1 opens up the opportunities that directed evolution could have when used on different cancer types and with a range of different viruses. Due to the high potency reported here, one consideration which should be noted is that restriction of replication tropism to cancer cells only needs to be attained to ensure off target toxicity is avoided [56].

As discussed previously, it has been shown that enhancing tumor selectivity of OVs through genetic modification can lead to enhanced efficacy and reduced adverse effects with more virions reaching and infecting target cells [57]. Gene silencing has been used to reduce viral spread to off target sites [58–62]. One example using the Semliki Forest virus reported a reduced viral spread to the central nervous system whilst maintaining glioma targeting in vivo through the introduction of neuron-specific micro-RNA (miRNA) sequences [63]. Another glioma-specific OV was reported by Delwar et al. in which HSV-1 was transcriptionally regulated using a specific survin promoter in place of the ICP4 gene leading to enhanced replication in gliomas when compared to normal neuronal cells ex vivo [64].

Genetic modification has also been used to alter virus capsid surface and thereby directly alter cell tropism [65,66]. Furthermore such modification of capsid surface can also coupled with chemical strategies, to enhance detargeting of OV to nontarget tissues and retarget them to both tumor tissues and their associated vasculature. Genetic modification of the oncolytic measles virus was performed to create a receptor specific, and hence infection specific, OV. Here the viral surface was modified to display single-chain antibody fragments against target receptors, including CD38 and epidermal growth factor receptor (EGF) [67]. Receptor-mediated infection was observed both in vitro and in vivo, leading to antitumor activity in vivo. In another interesting approach, Kreppel et al. [68] genetically modified the capsid of Ad5 to introduce cysteine residues into the HI-loop of the fiber protein. These cysteine residues were then conjugated via a bifunctional polymer to transferrin molecules while the rest of the capsid surface was modified with polyethyleneglycol N-succinimidyl propionate (SPA-PEG). This resulted in an Ad vector which was uniquely selective for cells with transferrin receptors and hence unable to infect tissues which were not desired targets in vivo. A combined genetic and chemical modification was also used on Ad5 to replace its naturally occurring coagulation factor X (FX) shield a change which was shown to reduce detection by NABs and complement in vitro, and extend circulation time and hepatocyte infection in vivo [69].

Choice of serotypes with low sero-prevalence in the human population (as with ColoAd1) is one approach to dampening blood stream neutralization. Notably, the wild-type Newcastle disease virus (NDV) isolated from Russian migratory birds, which is nonpathogenic in mammals, has been shown to selectively replicate in malignant cells [12]. NDV is unable to replicate in healthy cells due to their functional interferon (IFN) response [70,71] but replicates rapidly in cancer cells with ablated IFN response thereby inducing cell death. NDV has been proved effective in preclinical studies and Phase I and II clinical trials have been performed [12,72–74]. In addition, some OV, such as the VV, have their own ‘natural’ methods to prevent detection whilst undergoing cell to cell infection. Once replication begins, VV generates two subspecies. One of these subspecies, the extracellular enveloped virus takes proteins from the membrane of the cell it has infected and uses them to form an envelope which has been shown to enable its avoidance of neutralizing antibodies [75].

2.1.2. Chemical Section 2.1.1 discussed some of the genetic modifications which have been used to enhance the delivery of OV. These strategies, although effective, can be expensive and complex, and can require new engineered and validated cell lines and harvesting procedures. This section provides details of some of the studies reported using theoretically simpler and less expensive chemical modification strategies to enhance the systemic delivery of OV.

2.1.2.1. Polymer stealling viruses for enhanced blood stability. The bloodstream stability and tumor accumulation of OV can be improved using biocompatible polymers to shield the virus from antibody binding and interactions with other blood components [8].

Biocompatible polymers are used in a wide range of drug delivery systems [76]. Such polymers are extremely useful carriers as they have good blood compatibility and maintain structural integrity. Polymer-coated therapeutic viruses were first reported in 1999 [77]. Polymer coating strategies most commonly utilize polymers, typically based on a PEG or poly[N-(2-hydroxypropyl) methacrylamide] (PHPMA) polymer backbone, to modify viruses covalently or noncovalently. Covalent methods have involved the use of monofunctional polymers with terminal amine reactive groups. These amine reactive
groups react with viral capsid lysine residues creating either brush-like or complete polymer shields [51,68,77–82]. These modifications of the viral capsid can lead to a knockdown, or in some cases ablation, of viral infectivity.

Early examples of polymer coating were able to provide the therapeutic viruses with protection from the immune system whilst maintaining a sufficient level of infection. In cases where infection ability was reduced or ablated, bifunctional polymers were used with targeting ligands attached to produce a retargeted therapeutic virus. This will be discussed further in Section 2.1.2.2.

Much of the work performed to characterize the impact of polymer coating on clearance, infection, and blood stability of OV was performed on viruses based on Ad. PEGylation of Ad5 has been reported to reduce binding of neutralizing antibodies (NAbs) both in vitro and in vivo [82,83]. Notably, it was also shown that PEGylation reduced anti-Ad5 adaptive immune response induction in immune competent mice with a reduction in the number of cytotoxic T lymphocytes (CTLs) and anti-NAbs detected, a finding which has implications for redosing regimes [83]. Interestingly, it was also shown that the reactive group used for the PEGylation impacted the scale of the NAbs response against Ad5 that was generated, with the PEGylation using a methoxypolyethylene glycol tresylate reactive group producing the lowest titre of NAbs. These PEGylated Ad5 were still able to express transgenes, and although this ability was still inhibited after repeat dosing in murine models [83], when sequential doses of Ad5 modified using different amine reactive linkers was used infectivity could be maintained. This raises the hypothesis that the NAbs produced were specific to the antigenic epitopes associated with the linker used. Heavy PEGylation of Ad5 was shown to reduce blood clearance rate fourfold [84] and experiments followed to show that despite a reduction in Kupffer cell capture, PEGylation strategies did not reduce hepatic damage in vivo [85]. PEGylation of Ad6 using a SPA linker showed that liver damage could be reduced for this serotype [86].

Fisher et al. compared the use of a multivalent hydrophilic polymer based on PHPMA to the use of PEG [51]. PHPMA was considered the preferred material for coating as it required a lower concentration of reactive esters for cooperative binding than PEG. Using transmission electron microscopy, it was determined that the polymer-coated Ad retained its gross morphology, and analysis of particle size using photon correlation spectroscopy showed a diameter increase of 23.1 nm. Infection of polymer-coated Ad in vitro was abolished because the PHPMA coating blocked the regions of the Ad used for binding [51]. A further study showed that while viral infectivity is reduced when Ad is modified with PHPMA, hepatic uptake was also reduced by almost 60% in comparison to an uncoated Ad [87]. Overall hepatic toxicity was also decreased, the impact of which means higher doses of coated viral particles could be safely administered [87]. The polymer-coated Ad had an increased circulation half-life (> 0 vs ~5 min) as a result of blocking receptor-mediated clearance and infection pathways [87]. A study followed which took advantage of this increase in circulation time which showed that increased passive tumor accumulation was obtained for PHPMAylated Ad5 [88]. When Kupffer cell inhibiting biophosphonate liposomes were preadministered followed by polymer-coated Ad, acute inflammatory toxicities related to Ad treatment were prevented [89]. This vector was able to avoid rapid clearance but incapable of infecting any cells, and so provided a platform onto which retargeting ligands could then be attached to provide specific cancer cell tropism (discussed further in Section 2.1.2.2).

This approach did produce some impressive findings but knockdown in infectivity and the lack of control of which epitopes were being modified were limitations. Studies utilizing ‘bioresponsive’ polymers (e.g. polymers that were cleavable in acidic conditions) to shield Ad were investigated [90,91]. After intratumoral injection, Carlisle et al. [91] showed increased levels of infection with a bioresponsive PHPMA coating when compared with an irreversible PHPMA Ad5 coating. The bioresponsive coating also achieved an 8000-fold increased in hepatic sequestration and a 50-fold increase in circulation time when compared with unmodified Ad5.

Noncovalent methods using cationic polymers (e.g. polyacrylates [92], branched polyamine copolymers [93], and polyglycerides [94–97]) to coat viruses were first reported in 1997 but achieved limited success due to their serum instability [98]. The modulation of complex surface charge using PEG or oligoethylene glycol blocks enabled the polymer-coated viruses to overcome their instability in serum. Kwon et al. [99] showed that a noteworthy $10^5$ increase in tumor to liver ratio could be achieved when Ad was modified with a PEGylated chitosan targeted to the folate receptor when compared with naked Ad. A further study reported the use a PEGylated copolymer for the noncovalent modification of Ad which showed the tumor-to-liver ratio increased by over 1000-fold compared to naked Ad in vivo which led to tumor growth suppression with minimal liver toxicity [95]. Furthermore, it was reported that the use of a bioresponsive cationic PEG for modification of Ad resulted in innate and adaptive immune response circumvention, negligible hepatotoxicity, and enhanced tumor growth suppression when compared with naked Ad [100]. To date, none of these preclinically promising Ad modification approaches have progressed into the clinics. This might reflect insufficient consideration of the differences in immune status and clearance pathways between animal models and humans and difficulties in scaling these coating approaches to the quality standards required for clinical use.

Whilst there has been a wealth of research into the chemical modification of nonenveloped viruses, little has been reported regarding chemical modification of enveloped viruses such as HSV and VV. One example of chemical modification by Nosaki et al. used layer-by-layer deposition of a linear polyethyleneimine hydrochloride (PEI) and negatively charged chondroitin sulfate to coat an oncolytic measles virus (MV) [101]. Modification was shown to protect MV from NAbs in vitro and in vivo. In vivo preimmunized mice bearing subcutaneous LL2-CD46 tumors were treated intratumorally with MV or modified MV and enhanced cell lysis was observed for modified MV.

Overcoming the challenges associated with systemic delivery of enveloped viruses has not however been completely overlooked. Studies have been performed into the use of cell
carrier systems for effective delivery of enveloped OVs and are discussed in Section 2.3. An alternative method involving the enzymatic treatment of the oncolytic VV surface to remove its N-linked and simple O-linked glycans, namely a deglycosylated oncolytic VV, was able to reduce neutralization by preexisting antibodies, and reduce TLR2 activation, and anti-VV NAbs production. This translated to an increase in viral gene expression after 24 h, increasing to a fivefold increase after 5 days, in vivo with deglycosylated oncolytic VV when compared with unmodified VV [102].

A final approach worthy of mention is the application of molecules to dampen the anti-OV immune response. Evgin et al. demonstrated that pretreatment with complement inhibiting peptide extended the pharmacokinetics and increased the activity of an oncolytic VV [50]. This study is an excellent demonstration of the barrier presented by complement. Translation into a clinical context with patients in varying degrees of ill-health and immunosuppression due to their underlying disease and the impact of previous failed therapies may be a complex process.

2.1.2.2. Active retargeting of oncolytic viruses. Active retargeting strategies have been employed in the delivery of a host of different types of cancer therapeutics. In common with the retargeting by genetic modification of viral capsid proteins [65], these techniques take advantage of cell-surface proteins that are over expressed in tumors and uses ligands to target them. Both large proteins (e.g. antibodies) and small peptides can be used as agents for active OV retargeting, decorating the surface of the virus which leads to increased tumor recognition and cell entry [103].

A seminal report of the addition of targeting peptides to a PEG modified Ad2 in 1999 by Romanczuk et al. [104] showed successful evasion of NAbs which led to a fourfold to fivefold increase in cancer cell infection in vitro. Fisher et al. [51] showed that once successfully detargeting of Ad5 using a PHPMA shield had been achieved, viral tropism could be redirected using fibroblast growth factor-2 (FGF2) or vascular endothelial growth factor-165 (VEGF165) to infect cancer cell lines with the corresponding receptors. As mentioned previously (Section 2.1.1), controlled modification of capsid proteins could lead to enhanced efficacy for modified OVs and this was explored in a study by Campos and Barry [105]. It was suggested using Ad that retargeting through the attachment of ligands might only be effective when done through the site on the OV which is responsible for natural tropism; i.e. the fiber protein in the case of Ad.

Initial translation of these successful retargeting studies in vivo did not merit huge success. Stevenson et al. reported that despite seeing strong selective infection of PC-3 cells through a laminin-derived (SIKVAV) peptide in vitro, there was no observed increase in transgene expression in vivo [80]. Green et al. reported similarly disappointing outcomes using an FGF2 ligand to retarget Ad and showed a decrease in tumor infection after intravenous administration in vivo, with the retargeted Ad having strong association with murine erythrocytes [78]. More positive results were seen by Morrison et al. but these studies were performed using intraperitoneal administration [79].

2.2. Improving delivery within solid tumors

One of the cell-death mechanisms utilized by OV is to infect and destroy cancer cells (oncolysis) via necrosis, releasing viral progeny after the cancer cells are killed [106]. These progeny OVs are then responsible for cell-to-cell spread and infection of surrounding cells so that efficient cell death is possible from a small initial delivered dose. However, there are limitations to the viral spread into and throughout tumors. Tumor physiology poses a formidable barrier in cancer treatment as its heterogeneous morphology results in unpredictable and nonuniform drug distribution [107]. During the delivery phase, OVs do not typically have a sufficiently long-circulation half-life to benefit greatly from the enhanced permeability and retention (EPR) effect, meaning only a tiny percentage of the IV-administered dose passively accumulates in tumors. As cancer treatment modalities continue to move away from the conventional small drug molecules toward larger biologics the selective passive accumulation of drugs at tumor sites due to tumor’s enhanced permeability will have less and less impact. The benefit gained from the EPR effect brought about by the leaky vasculature found in tumors [108,109] has been heavily relied on but has shown to be unable to enhance delivery of a range of anticancer therapeutics (including small molecule drugs, antibodies, and OVs) over distances of more than around 50 µm from the vasculature [91,110–112]. This means a small amount of virus can infect and spread near the blood vessels which supply a tumor, but the majority of the tumor remains untreated. In response, research has been directed toward developing mechanically activated transport mechanisms to aid the penetration of OV and hence increase the anticancer activity of viruses.

2.2.1. Modification of intratumoral physiology

Whilst the challenge of poor bloodstream stability and rapid clearance may be best addressed by trying to alter the OV vectors (by genetic or chemical means or both). The challenge of poor penetration into and throughout target tumors may be best addressed by trying to alter the tumor environment.

2.2.1.1. Vascular normalization. Angiogenesis, the development of new blood vessels, is crucial for healthy human growth [113]. In healthy adults, angiogenesis is a heavily regulated process, triggered by specific molecular and mechanical stimuli. Many diseases are characterized by the uncontrolled formulation of new blood vessels, including solid tumors. Induction of new vessel formulation is stimulated by a huge range of molecular features within the tumor; angiogenesis is relentless [114–116]. The result is a microvascular network which is able to produce a rich blood supply, but which is very abnormal and disorganized. Indeed some areas of the tumor have excessive blood flow whilst others have next to no perfusion [116–119].

Vascular endothelial growth factor (VEGF) is the driving force behind tumor angiogenesis and a host of investigations using inhibitors of the VEGF receptor as monotherapies to ameliorate VEGF activity have been performed. Although these studies provided strong preclinical data [120], clinical results have been less encouraging. In response, more
sophisticated combination regimens have been developed, with the concept of vascular normalization being applied [121]. Vascular normalization uses antiangiogenic drugs to prune some vessels and transform the abnormal vessel structure into a more normal state [113].

Specifically, anti-VEGF/VEGFR therapy has been associated with successfully reducing vessel density, vessel maturation and, in some cases, reorganization of the basement membrane [122–124]. With these structural changes to the vasculature, normalization of the vessel function also occurs. A reduction in vessel permeability brought about with therapy against the VEGF-VEGFR pathways leads to a reduction in IT interstitial fluid pressure. This reestablishes a pressure gradient across the vasculature and improves perfusion which enables improved drug penetration in tumors [123–125]. Such vascular modulation has generated interest in the field of small molecule delivery, but to date has been under-researched for OV delivery.

Recently, it was shown in vivo that oncolytic VV-targeted tumor blood vessels in pancreatic neuroendocrine tumors in RIP-Tag2 transgenic mice which led to vascular pruning and elongated viral leakage in tumors. Of interest, this effect was not inhibited by VEGFR2 inhibition [126]. An in vivo study by Kurozumi et al. showed that IT administration of the OV hrR3 (a derivative of the HSV-1) had antiangiogenic effects resulting in significantly greater vascular leakage when compared with PBS treatment in mice with intracranial D74/HveC glioma cell tumors [127]. This was investigated by imaging of resected brain tumor sections from treated mice, injected with Texas Red-dextran 5 min before cull. The addition of an angiogenic inhibitor, cRGD peptide, treatment prior to OV IT administration to reduce vascular permeability was also investigated. Mice were treated with either cRGD or PBS 3 days after intracranial cell implantation, and were then randomized and treated with OV 7 days after implantation. The resected brain tumor sections showed significantly reduced vascular density when treated with cRGD over those treated with PBS. The effect of the reduction of perfused functional vessels was then supported by the reduction in the number of tumor blood vessels seen using immunostaining with anti-CD31, an antibody specific for endothelial cells [127]. Furthermore, it was shown that pretreatment with cRGD reduced microvessel density and prevented rapid viral clearance from the tissue, which led to significantly increased survival of mice pretreated with cRGD and then given IT OV therapy [127].

Following this report that hrR3 has antiangiogenic properties itself, the same observation was reported by Breitbach et al. who showed in humans that VV disrupts tumor-associated vasculature after intravenous administration. The selective infection of tumor-associated endothelial cells caused reduced perfusion and increased hypoxia in the tumor as early as 5 days after treatment [128]. This was further reported by Hou et al. who observed that revascularization after treatment with VV was delayed until after it was cleared in murine models [129]. It is doubtful whether such events could ever be controlled sufficiently to enable vascular normalization created by a first-dose OV to enhance subsequently delivered chemotherapy. Indeed, the concept of vascular normalization to enhance subsequently IV-dosed OV, may be floored, as work with liposomes suggests that any gains provided by reestablishment of tumor perfusion are negated by decreased passage of particulates through vasculature that has regained structural integrity and lost EPR [111]. However, studies using tumor necrosis factor alpha (TNF-α) have shown that this modifier of vascular permeability may well provide a good route to enhanced tumor uptake of OV when used in a perfused limb context [130, 131]. The differences in the timescales and modes of action between anti-VEGF-based and TNF-α-based perfusion modulation may explain the opposing impact they have when combined with OV, although safe systemic use of TNF-α remains a challenge. Of note, a study by Arulanandam et al. reported that delivery and spread of IV-administered oncolytic VV was inhibited when VEGF signaling was supressed in vivo, further questioning whether combined sequential vascular normalization treatment followed by OV infection is suitable [132].

### 2.2.1.2. Altering the internal pressure gradient

Studies have been performed to address whether actively altering the pressure gradient across a tumor can enhance transvascular OV transport. One impressive and important study by Miller et al. [133] looked at the interaction of blood pressure on blood flow and tumor perfusion in multiple myeloma tumor models. This study compared mice in which the mean arterial pressure (MAP) was manipulated to either increase or decrease the IT perfusion pressure. They showed that physical exercise could be used as a method to increase the mouse MAP, ~160 mm Hg, and inhalation of 5% isoflurane would decrease the mouse MAP, 50 mm Hg. Vesicular stomatitis virus (VSV) was then delivered systemically to mice bearing myeloma tumors [133]. Miller et al. showed using SPECT/CT imaging that there was significantly increased density of VSV IT infection on days 1 and 2 posttreatment in the exercise group. Tumors harvested on day 1 posttreatment showed around a 100-fold increase in VSV present in the exercise group over those present in the 5% isoflurane group. This increased infection resulted in significantly higher survival in the exercise (high MAP) group when compared to the 5% isoflurane (low MAP) group. Crucially, the survival in the 5% isoflurane group was not statistically significant compared to the saline control group [133]. It has also been noted that the findings in this study, showing the impact of anesthesia on IT pressure and hence perfusion in murine models, provides strong evidence for the need for standardized animal handling and anesthesia protocols [134]. It has been suggested that difference in these practices could be a reason why there has been variability in delivery and efficacy of similar therapeutics used in different facilities.

### 2.2.2. Ultrasound

Ultrasound (US) is attractive for use as a therapeutic delivery mechanism as it has been utilized for many years in the biomedical imaging field and hence has a well-defined safety profile [135]. Therapeutic US has a wide range of applications including thermal ablation of tissue, lithotripsy, enhanced
drug delivery, temporary opening of the blood brain barrier, and triggered drug release [136]. US can be focused to within a few cubic millimeters and is thus can provide a pinpoint accurate external stimulus, especially when used to create cavitation events.

2.2.2.1. Acoustic cavitation. The positive and negative peaks in pressure created by an of an US wave impact upon gas bubbles in its path. These may be naturally entrained bubbles or bubbles provided by injection of micro- or nanobubble formulations [137]. During the alternating rarefactual and compressional cycles of the US wave, the bubble may expand and collapse in a stable fashion. However, if the pressure is sufficient, the bubble will expand and then collapse under the inertia of the surrounding media. This is known as inertial cavitation. During cavitation events, there are two mechanisms which increase blood flow; acoustic streaming and microstreaming [138]. The streaming mechanisms and shock waves that are generated by cavitation have the potential for use in enhancing drug delivery to tumors [139]. It is believed that microstreaming, caused by cavitation, is the overarching force which delivers therapeutic agents deep into solid tumors and can facilitate the delivery of OV from the bloodstream [135].

2.2.2.2. Ultrasound-mediated cavitation for enhanced drug delivery. The earliest report of enhanced delivery of OV using US was in 2006 [140]. Adenoviral vectors were incubated in the presence or absence of microbubbles before being incubated with human plasma and exposed to 2.5 MHz of US at 535 kPa continuously for 1 min in the presence of DU-145 and H23 cells. Ad-GFP-microbubble formulations showed a strong fluorescence signal when infection took place with US exposure when compared with the same formulation in the absence of US exposure [140].

An early hallmark study was reported in 2010 [141] when enhanced delivery of oncolytic Ad microbubble constructs (Ad-MB) was seen in the US-treated tumor in one of two bilateral tumors in the same mouse. A further study also showed that in two xenograft mice models, enhanced delivery in one tumor led to growth reduction in the other tumor. This could suggest that increased tumor infection in one tumor led to systemic abscopal antitumor effects; however, it is noted that a lack of an adequate control for just intravenously injected Ad-MB limits the conclusions that could be made [141].

Increased OV delivery into and improved dissemination through tumors was shown using a polymer-stealthed Ad and US-induced cavitation [91]. Ad was ‘stealthed’ with a PHPMA polymer, showing a ~ 50-fold increase in circulation half-life in murine models bearing human ZR-75-1 xenograft tumors (see Section 2.1.2). To noninvasively increase extravasation of the circulating polymer-coated Ad into the tumor, it was coinjected with gas microbubbles and the tumor was exposed to 0.5 MHz focused US at peak rarefactual pressure of 1.2 MPa, triggering inertial cavitation which was then monitored remotely in real time [91]. The combination of polymer stealthing and US-induced cavitation resulted in a significant increase in tumor infection, measured by bioluminescence, of over 30-fold ($p = 0.03$) [91]. Not only was an increased in tumor infection seen, but dissemination into the tumors away from the blood vessels was also improved, with cell death seen hundreds of microns from blood vessels, compared to tens of microns without US.

Microbubbles are destroyed in the process of undergoing inertial cavitation meaning that the cavitation events provided are rather short-lived, and repeat microbubble administration is required if cavitation levels sufficient to provide enhanced delivery are to be achieved [142]. Kwan et al. have addressed this limitation with the development of a nanoscale sonosensitive particle (SSP) which is capable of trapping sustaining cavitation for several minutes [142], substantially longer than the cavitation time observed with microbubbles.

A recent study [143] saw this nanoscale cavitation technology applied in vivo to OV delivery. It was reported that when a combined IV injection of SSVs and luciferase expressing VV were administered in CD1 nude mice bearing xenograft HepG2 or SKOV-3 tumors and exposed to focused US there was a substantial (1000- to 10,000-fold) increase in infectivity. This increase of infectivity correlated with number of VV genomes recovered from the tumors as assessed by qPCR at cull. Furthermore, this study looked into the comparison of a commercially available US contrast agent (UCA), SonoVue® (SV), and showed significantly improved infection using SSP ($p < 0.05$).

The use of therapeutic US for enhanced delivery of OV therefore offers a potential solution to the reported limited tumor extravasation and IT viral spread.

2.2.3. Magnetic targeting and penetration

Magnetic drug targeting involves magnetic particles being loaded with the desired drug and then actively accumulated at the targeted delivery site using magnetic force. It is an attractive targeting technique as it is noninvasive and magnetic fields are able to pass through human tissue without attenuation [144]. The use of magnetic drug targeting of viruses was first reported in the early 2000 where retroviral nucleic acid delivery in vitro to human and murine cells was enhanced magnetically [145]. Shortly after Scherer et al. showed that Ad associated with paramagnetic particles showed a drastic increase in transfection efficiency in vitro in the presence of a magnetic field [146] and Mah et al. demonstrated magnetically enhanced transfection using a recombinant adeno-associated virus [147]. As different types of modified magnetic nanoparticles became readily available commercially, a wealth of papers reporting that magnetic modifications of lentivirus, Ad, and baculovirus led to improved infection in vitro were published [148–153].

Despite the numerous publications on magnetic drug targeting of viruses in vitro, there are few which have shown increased delivery in vivo. In 2009, oncolytic Ad di520 (Ad520) associated with magnetic nanoparticles at a ratio of 5 fg of Fe per virus particle and above and showed a 10-fold increase in viral uptake in multidrug resistant and CAR-deficient tumor cells compared to nonmagnetic oncolytic Ad in the presence of a magnetic field [154]. This increased viral uptake led to a 10-fold enhancement of oncolytic potency (IC$_{50}$ value) and four orders of magnitude more viral progeny. This effect was further investigated in pilot study in vivo and
showed increased tumor growth was suppressed after IT injection of magnetic AdS20 in the presence of a magnetic field in mouse tumor xenografts from human pancreatic carcinoma cells [154].

Most recently a patent filed by Zhu et al. describes increased liver accumulation in mice after a systemically injected baculovirus vector (expressing luciferase) conjugated to magnetic nanoparticles (MNP-BV) when a magnetic field was applied for 60 min [155]. Bioluminescence imaging showed high levels of detection in the liver only for the MNP-BV plus magnetic field group and undetectable levels in the kidney, heart, spleen, and lungs.

Magnetically guided drug delivery and targeting is often questioned for its clinical relevance due to the limitations in magnetic field strength at applicability at tissue depths. Delivery of magnetic agents in vivo has been shown within a ~20 mm depth from the magnet surface [156–159]; however, the magnetic force falls off very rapidly and beyond a depth of 50 mm, the magnetic field gradient required to produce an adequate magnetic force would be extremely large [160].

Magnetic targeting would, therefore, be best suited for subcutaneous tumors, such as melanoma, which are already effectively treated using IT injections of Imlygic®.

### 2.3. Cell carriers

One approach to address both blood clearance and tumor penetration issue with OV, is the use of cell carriers. The insertion of OV into ‘cell carriers’ ex vivo is a mechanism which has been explored both preclinically and clinically to enhance the delivery of OV [161]. In fact, a study administering naked reovirus to patients intravenously showed that all viral particles detected in the blood were cell-associated and hence, that cell carrier delivery may occur naturally for some viruses. Despite the proportion of this cell bound virus that ultimately deposits in the tumor being unknown, this study does demonstrate that cells can act as OV carriers [162]. Intriguingly, it was also shown that when human monocytes were loaded with preformed reovirus-antibody complexes (neutralizing the reovirus) they were able to deliver fully replication competent reovirus to tumor cells in vitro. In vivo administration of neutralized reovirus-antibody complexes were also shown to slow B16 mouse melanoma tumor growth compared to vehicle only [163]. A range of cell types have been used as cell carriers for variety of OV and the preclinical success of these have been well reviewed by Roy et al. [164] Determining which cell is best to use for different tumor indications is an important consideration when implementing carrier cell systems. Arguably, the leading candidate for cell carriers in OV delivery to tumors are stem cells. In vitro and in vivo studies using stem cells have been shown to allow viral infection and replication, evasion of immune detection, and provision of tumor tropisms [165,166]. Stem cells carrying viral particles are able to evade detection by antibodies and T cells as there are such a low number of stem cell antigen processing transporters [167]. The use of stem cells for in vivo treatment of cancer has shown much promise for a range of tumor models [168]. The results of an ongoing Phase I/II study (NCT02068794) administering mesenchymal stem cells infected with oncolytic measles virus will shed light on the translational ability for stem cell carriers in clinical use. Despite this method showing promise, there are obvious limitations for the use of cell carriers in therapy, with the main considerations being the need for ex vivo infection of cells and cost, manufacture, scalability, and regulatory requirements involved in bringing two very complex biologic therapeutics into one product.

### 3. Conclusion

OVs have shown impressive efficacy in preclinical and clinical settings but their potential can be restricted by antiviral neutralization in the bloodstream and inefficient delivery into the complex tumor environment. As a result a surge of research into enhancing the delivery of OV has occurred. Currently for many viruses, the issue of optimal delivery has been avoided by administrating OV locally. However, as more OV become clinically approved competition will drive the desire for wider clinical utility and larger treatable patient populations. As a starting point, the successful delivery of OV systematically will enable the treatment of patients with both solid tumors and metastatic cancer.

### 4. Expert opinion

In order for the field of oncolytic virotherapy to gain traction and covert potential into value, researchers were impelled to focus on showing clinical and commercial viability following IT injection into cancer types amenable to this administration route. Indeed, the approval of the Amgen product Imlygic in 2015 for use following IT injection into melanoma provided a great boon for the field and encouraged big pharma competitors to support OV programs [169–172]. However, although the range of OV being assessed in clinical trials has expanded [173], in the majority of cases delivery is still restricted to IT injection, an inefficient and hard to standardize route. If the technology is now to mature into one which can be applied across a wider range of patients with a wider range of cancer indications, the next hurdle to overcome will be to ensure that OV are amenable to systemic delivery. This may be achieved using strategies that modulate the OV by genetic or chemical means or that alter the physiology of target tumors, or a combination of all these approaches. However, challenges remain in translating the raft of powerful technologies which show good preclinical data into clinical practice. Viral modification through either/both chemical and genetic methods may offer a route to overcoming the greatest barriers to clinical adoption but negotiating regulatory approval will be challenging for these approaches and they will also face difficulties relating to the maintenance of specific and reliable modification during large-scale manufacture. For chemically modified OV ensuring their stability over longer storage times will also need to be addressed. Technologies focused on combining OV delivery with adapting the IT environment (e.g. the use of anesthetic and vascular normalization to reduce interstitial pressure) will likely have the easiest
transition into clinical practice as these techniques are currently being used in combination with other cancer therapeutics.

For this field to progress, the most advanced of the preclinically promising approaches and technologies described here will need to be tested in early-stage clinical trials. When the scale of the aforementioned manufacturing and regulatory challenges is balanced against the current commercial interest and potential reward of achieving a fully targetable and IV compatible OV, we predict that within the next 5 years clinical testing of chemical and/or genetic modification strategies will be underway.

Funding

This paper was funded by the Oxford Centre for Drug Delivery Devices and Research Councils UK - Engineering and Physical Sciences Research Council (1800926).

Declaration of interest

The authors were supported by the Oxford Centre for Drug Delivery Devices. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

A reviewer on this manuscript has disclosed that they are a cofounder of Turnstone Biologics, a company that is developing oncolytic viruses to be used as systemically delivered therapeutics. Peer reviewers on this manuscript have no other relevant financial relationships or otherwise to disclose.

References

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

1. Side effects of chemotherapy. Cancer.Net. [Internet]. [cited 2019 Feb 26]. Available from: https://www.cancer.net/navigating-cancer-care/how-cancer-treated/chemotherapy/side-effects-chemotherapy
2. Firdos M, Ziaddin DLB. Viral Therapy of Cancer. In: Harrington Ville P, editor. Chapter 9: oncolytic vaccinia. John Wiley & Sons, Ltd; 2008:151–169.
3. Kelly E, Russell SJ. History of oncolytic viruses: genesis to genetic engineering. Mol Ther. 2007 Apr;15:651–659. Available from http://linkinghub.elsevier.com/retrieve/pii/S1525001616313314
4. Martuza RL, Malick A, Markert JM, et al. Experimental therapy of human glioma by means of a genetically engineered virus mutant. Science. 80-; 1991:252:854–856. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1851332
5. Russell SJ, Peng K-W, Bell JC. Oncolytic virotherapy. Nat Biotechnol. 2012;30:658–670. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22781695
6. Lawler SE, Speranza M-C, Cho C-F, et al. Oncolytic viruses in cancer treatment. JAMA Oncol. 2017;3:841. Available from: http://oncology.jamanetwork.com/article.aspx?doi=10.1001/jamaoncol.2016.2064
7. Russell SJ, Peng K-W. Oncolytic Virotherapy: A Contest between Apples and Oranges. Mol Ther. 2017;25:1107–1116. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28392162
8. Cattaneo R, Miest T, Shashkova EV, et al. Reprogrammed viruses as cancer therapeutics: targeted, armed and shielded. Nat Rev Microbiol. 2008;6:529–540. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3947522/
9. Heise C, Sampson-Johannes A, Williams A, et al. ONX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nat Med. 1997;3:639–645. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9176490
10. Bell JC, Stojdl DF, Lichty B, et al. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. Nat Med. 2000;6:821–825. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10888934
11. Herrnston T. Gene delivery from replication-selective viruses: armoring guided missiles in the war against cancer. J Clin Invest. 2000;105:1169–1172. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10791988
12. Yurchenko KS, Zhou P, Kovner AV, et al. Oncolytic effect of wild-type Newcastle disease virus isolates in cancer cell lines in vitro and in vivo on xenograft model. Ulason leditor. PLoS One. 2018;13:e0195425. Available from: https://dx.plos.org/10.1371/journal.pone.0195425
13. Igney FH, Krammer PH. Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer. 2002;2:277–288.
14. Markert JM, Medlock MD, Rabkin SD, et al. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant gloma: results of a phase I trial. Gene Ther. 2000;7:867–874. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10845725
15. Markert JM, Liechty PG, Wang W, et al. Phase Ib trial of herpes simplex virus mutant G207 inoculated pre-and post-tumor resection for recurrent GBM. Mol Ther. 2009;17:199–207. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18957964
16. Harrington KJ, Hingorani M, Tanay MA, et al. Phase I/II study of oncolytic hsvgm-csf in combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell cancer of the head and neck. Clin Cancer Res. 2010;16:4005–4015. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20670951
17. Senzer NN, Kaufman HL, Amatruda T, et al. Phase II clinical trial of a granulocyte-macrophage colony-stimulating factor-encoding, second-generation oncolytic herpesvirus in patients with unresectable metastatic melanoma. J Clin Oncol. 2009;27:5763–5771. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19884534
18. Kaufman HL, Kim DW, DeRaffele G, et al. Local and distant immunity induced by intraluminal vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma. Ann Surg Oncol. 2010;17:718–730. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19915919
19. Conry RM, Khazaie ME, Saleh MN, et al. Phase I trial of a recombinant vaccinia virus encoding carinoembryonic antigen in metastatic adenocarcinoma: comparison of intradermal versus subcutaneous administration. Clin Cancer Res. 1999;5:2330–2337. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10499601
20. Mastrandolo MJ, Maguire HC, Eisenlohr LC, et al. Intratumoral recombinant GM-CSF-encoding virus as gene therapy in patients with cutaneous melanoma. Cancer Gene Ther. 1999;6:409–422. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10505851
21. B-H P, Hwang T, T-C L, et al. Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial. Lancet Oncol. 2008;9:533–542. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18495336
22. Hwang T-H, Moon A, Burke J, et al. A mechanistic proof-of-concept clinical trial with JX-594, a targeted multi-mechanistic oncolytic poxvirus, in patients with metastatic melanoma. Mol Ther. 2011;19:1913–1922. Available from: http://dx.doi.org/10.1038/mt.2011.132/nature06264
23. Zeh HJ, Downs-Canner S, McCart JA, et al. First-in-man study of western reserve strain oncolytic vaccinia virus: safety, systemic spread, and antitumor activity. Mol Ther. 2015;23:202–214.
24. Cripe TP, Ngo MC, Geller JI, et al. Phase 1 study of intratumoral pexa-vec (JX-594), an oncolytic and immunotherapeutic vaccinia virus, in pediatric cancer patients. Mol Ther. 2015;23:602–608. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1525901616300727

25. Mansfield D, Kyla J, Rosenfelder N, et al. Oncolytic vaccinia virus as a vector for therapeutic sodium iodide symporter gene therapy in prostate cancer. Gene Ther. 2016;23:357–368.

26. Breitbach CJ, Burke J, Jonker D, et al. Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. Nature. 2011;477:99–102. Available from: http://dx.doi.org/10.1038/nature10358

27. Liikanen I, Ahtiainen L, Hirvinen ML, et al. Oncolytic adenovirus with temozolomide induces autophagy and antitumor immune responses in cancer patients. Mol Ther. 2013;21:1212–1223. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23546299

28. Morse MA, Chaudhry A, Gabbitzsch ES, et al. Novel adenoviral vector induces T-cell responses despite anti-adenoviral neutralizing antibodies in colorectal cancer patients. Cancer Immunol Immunother. 2013;62:1293–1301. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23624851

29. Nemunaitis J, Nemunaitis J. Head and neck cancer: response to p53-based therapeutics. Eisele DW, editor. Head Neck. . 2011;33:131–134. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20222046

30. Chang J, Zhao X, Wu X, et al. A Phase I study of KH901, a conditionally replicating granulocyte-macrophage colony-stimulating factor: armed oncolytic adenovirus for the treatment of head and neck cancers. Cancer Biol Ther. 2009;8:676–682. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19242097

31. Li J-L, Liu H-L, Zhang X-R, et al. A phase I trial of intratumoral administration of recombinant oncolytic adenovirus overexpressing HSP70 in advanced solid tumor patients. Gene Ther. 2009;16:376–382. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19090289

32. Barton KN, Paielli D, Zhang Y, et al. Second-generation replication-competent oncolytic adenovirus armed with improved suicide genes and adp gene demonstrates greater efficacy without increased toxicity. Mol Ther. 2006;13:347–356. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16290236

33. Reid TR, Freeman S, Post L, et al. Effects of Onyx-015 among metastatic colorectal cancer patients that have failed prior treatment with 5-FU/leucovorin. Cancer Gene Ther. 2005;12:673–681. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15803147

34. Morley S, MacDonald G, Kim D, et al. The d1520 virus is found preferentially in tumor tissue after direct intratumoral injection in oral carcinoma. Clin Cancer Res.. 2004;10:4357–4362. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15240522

35. Hecht JR, Bedford R, Abbruzzese JL, et al. A phase I/II trial of intratumoral endoscopic ultrasound injection of ONX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma. Clin Cancer Res.. 2003;9:555–561. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12576418

36. Nemunaitis J, Cunningham C, Buchanan A, et al. Intravenous infusion of a replication-selective adenovirus (ONX-015) in cancer patients: safety, feasibility and biological activity. Gene Ther. 2001;8:746–759. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11420638

37. Sheridan C. First Oncolytic virus edges towards approval in surprise vote. Nat Biotechnol. [Internet]. 2015;33:569–570. Available from: http://www.nature.com/doifinder/10.1038/nbt.3190

38. Garber K. China approves world’s first oncolytic virus therapy for cancer treatment. JNCI J Natl Cancer Inst. [Internet]. 2006 [cited 2019 Jan 23];98:298–300. Available from: http://academic.oup.com/jnci/article/98/5/298/2522047/China-Approves-Worlds-First-Oncolytic-Virus

39. Liu BL, Robinson M, Han ZQ, et al. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Ther; 2003;10:292–303.

40. Bommareddy PK, Patel A, Hossain S, et al. Talimogene Laherparepvec (T-VEC) and Other Oncolytic Viruses for the Treatment of Melanoma. Am J Clin Dermatol. 2017;18:1–15. Available from: http://link.springer.com/10.1007/s40257-016-0238-0

41. Rehm H, Silk AW, Kane MP, et al. Into the clinic: talimogene laherparepvec (T-VEC), a first-in-class intratumoral oncolytic viral therapy. J Immunother Cancer. 2016;4:53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27660707

42. Andtbacka RHI, Kaufman HL, Collicchio F, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol. 2015.

43. Selding KA, Barry RD, Shafren DR. Enhanced oncolysis mediated by Coxackievirus A21 in combination with doxorubicin hydrochloride. Invest New Drugs. 2012;30:568–581. Available from: http://link.springer.com/10.1007/s10637-010-9614-0

44. Andtbacka RHI, Curti BD, Kaufman H, et al. CALM study: A phase II study of an intratumorally delivered oncolytic immunotherapeutic agent, coxsackievirus A21, in patients with stage IIC and stage IV malignant melanoma. J Clin Oncol. 2014;32:3031. Available from: http://ascopubs.org/doi/10.1200/jco.2013.32.15_suppl.3031

45. Bernstein V, Ellard SL, Dent SF, et al. A randomized phase II study of weekly paclitaxel with or without palereop in patients with metastatic breast cancer: final analysis of Canadian Cancer Trials Group IND.213. Breast Cancer Res Treat. 2018;167:485–493. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29027598

46. Samson A, Scott KJ, Taggart D, et al. Intravenous delivery of oncolytic reovirus to brain tumor patients immunologically primes for subsequent checkpoint blockade. Sci Transl Med. 2018;10:eaaam7577. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29298869

47. Heo J, Reid T, Ruo L, et al. Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia virus JX-594 in liver cancer. Nat Med. 2019;25:329–336.

48. Jennerex TSA. Jennerex and transgene present positive clinical data from phase 2 trial of JX594/TG6006 in sorafenib-refractory liver cancer patients [Internet]. [cited 2017 Aug 6]. Available from: https://www.transgene.fr/wp-content/uploads/PR214_en.pdf.

49. Downs-Canner S, Guo ZS, Ravindrathan R, et al. Phase 1 study of intravenous oncolytic poxvirus (vvDD) in patients with advanced solid cancers. Mol Ther. 2016;24:1492–1501. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/mt/journal/vaop/ncurrent/full/mt2016101a.html

50. Evgin L, Acuna SA, Tanese de Souza C, et al. Complement inhibition prevents oncolytic vaccinia virus neutralization in immune humans and cynomolgus macaques. Mol Ther. 2015;23:1066–1076. [Internet]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25807289

51. Fisher KD, Stallwood Y, Green NK, et al. Polymer-coated adenovirus permits efficient retargeting and evades neutralising antibodies. Gene Ther. 2001;8:341–348.

52. Miller A, Suksanpaisan L, Naik S, et al. Reporter gene imaging identifies intratumoral infection voids as a critical barrier to systemic oncolytic virus efficacy. Mol Ther Oncolytics. 2014;1:14005. [Internet]. Available from: http://dx.doi.org/10.1038/mt.2014.5

53. Chen DS, Millman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39:1–10.

54. Bourgeois-Daigneault M-C, Roy DG, Aitken AS, et al. Neoadjuvant oncolytic virotherapy before surgery sensitizes triple-negative breast cancer to immune checkpoint therapy. Sci Transl Med. 2018;10:eaaao1641. Available from http://stm.sciencemag.org/

55. Kuhn I, Harden P, Banzon M, et al. Directed evolution generates a novel oncolytic virus for the treatment of colon cancer. Jin D-Y, editor. PLoS One. 2008;3:e2409. Available from: https://dx.plos.org/10.1371/journal.pone.0002409

56. Banzon M, Hermiston TW. Oncolytic viruses: the power of directed evolution. Adv Virol. 2012;2012:586389. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22312363

57. Patel MR, Kratzke RA. Genetic engineering of oncolytic viruses for cancer therapy. transl. Gene Ther to Clin. 2015;261–279. Available
C. HILL AND R. CARLISLE

from: https://www.sciencedirect.com/science/article/pii/B978012805637000178

58. Leber MF, Bossow S, Leonard VHJ, et al. MicroRNA-sensitive oncolytic herpes simplex virus-1 for selective killing of prostate cancer cells. Clin Cancer Res. 2009;15:5126–5135.

59. Hikichi M, Kidokoro M, Haraguchi T, et al. MicroRNA regulation of glycoprotein B5R in oncolytic vaccinia virus reduces viral pathogenicity without impairing its antitumor efficacy. Mol Ther. 2011;19:1107–1115.

60. Lee CYF, Rennie PS, Jia WWG. MicroRNA regulation of oncolytic herpes simplex virus-1 for selective killing of prostate cancer cells. Clin Cancer Res. 2009;15:5126–5135.

61. Edge RE, Falls TJ, Brown CW, et al. A let-7 microRNA-sensitive vesicular stomatitis virus demonstrates tumor-specific replication. Mol Ther. 2008;16:1437–1443.

62. Kelly EJ, Hadac EM, Greiner S, et al. Engineering microRNA responsiveness to decrease virus pathogenicity. Nat Med. 2008;14:1278–1283.

63. Ylosmaki E, Martikainen M, Hinkkanen A, et al. Attenuation of semliki forest virus neurovirulence by microRNA-mediated detargeting. J Virol. 2013;87:335–344. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23077310.

64. Delwar ZM, Liu G, Kuo Y, et al. Tumour-specific triple-regulated oncolytic herpes virus to target glioma. Oncotarget. 2016;7:28658–28669. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27070093.

65. Verheije MH, Rottier PJM. Retargeting of viruses to generate oncolytic agents. Adv Virol. 2012;2012:798526. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22312365.

66. Petrovic B, Gianni T, Gatta V, et al. Insertion of a ligand to HER2 in gB retargets HSV tropism and obviates the need for activation of the other entry glycoproteins. Palese P, editor. PLoS Pathog. 2017;13:e1006352. Available from: https://dx.plos.org/10.1371/journal.ppat.1006352.

67. Nakamura T, Peng K-W, Harvey M, et al. Rescue and propagation of fully retargeted oncolytic measles viruses. Nat Biotechnol. 2005;23:209–214. Available from: http://www.nature.com/articles/ntb01060.

68. Kreppel F, Gackowski J, Schmidt E, et al. Combined genetic and chemical capsid modifications enable flexible and efficient de- and retargeting of adenovirus vectors. Mol Ther. 2005;12:107–117. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15963926.

69. Krutzke L, Prill JM, Engler T, et al. Substitution of blood coagulation factor X-binding to Ad5 by position-specific PEGylation: preventing vector clearance and preserving infectivity. J Control Release. 2016;235:379–392. Available from: https://www.sciencedirect.com/science/article/pii/S016836591630387X?via%3Dihub.

70. Reichard KW, Lorence RM, Cascino CJ, et al. Newcastle disease virus mediated interferon response determines the outcome of newcastle disease virus infection in normal and tumor cell lines. J Virol. 2001;75:4792–4801.

71. Alemany R, Suzuki K, Curiel DT. Blood clearance rates of adenovirus type 5 in mice. J Gen Virol. 2000;81:2605–2609.

72. Freeman AI, Zakay-Rones Z, Gomori JM, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma patients. J Clin Oncol. 2011;29:1900–1907.

73. Prill JM, Krischum N, Zhang Y, et al. PE Gyelation of E1-deleted adenovirus vectors allows significant gene expression on readministration to liver. Hum Gene Ther. 2002;13:1887–1900.

74. Wongsanan P, Croyle MA, PEGylated Adenoviruses: from Mice to Monkeys. Viruses. 2010;2:468–502. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21994645.

75. Croyle MA, Chirmule N, Zhang Y, et al. Stealth adenoviruses blunt cell-mediated and humoral immune responses against the virus and allow for significant gene expression upon readministration in the lung. J Virol. 2001;75:4792–4801.

76. Mok H, Palmer DJ, Ng P, et al. Evaluation of polyethylene glycol modification of first-generation and helper-dependent adenoviral vectors to reduce innate immune responses. Mol Ther. 2005;11:66–79. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1525001604014637.

77. Nguyen TV, Heller GJ, Barry MAME, et al. Evaluation of polymer shielding for adenovirus serotype 6 (Ad6) for systemic virotherapy against human prostate cancers. Mol Ther - Oncolytics. 2016;3:15021. Available from: https://www.sciencedirect.com/science/article/pii/S1523777016300008.

78. Green NK, Herbert CW, Hale SJ, et al. Extended plasma circulation time and decreased toxicity of polymer-coated adenovirus. Gene Ther. 2004;11:1256–1263. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15215884.

79. Fisher KD, Green NK, Hale A, et al. Passive tumour targeting of polymer-coated adenovirus for cancer gene therapy. J Drug Target. 2007;15:546–551. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17671901.

80. Green NK, Hale A, Cawood R, et al. Tropism ablation and stealthing of oncolytic adenovirus enhances systemic delivery to tumors and improves virotherapy of cancer. Nanomedicine. 2012;7:1683–1695. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22709345%5Cnhttp://www.futuremedicine.com/doi/abs/10.2217/nmm.12.50.

81. Prill JM, Subr V, Pasquarelli N, et al. Traceless bioresponsive shielding of adenovirus hexon with HPMA copolymers maintains transduction capacity in vitro and in vivo. Hong S-S, editor. PLoS One. 2014;9:e82716. Available from: https://dx.plos.org/10.1371/journal.pone.0082716.

82. Carlini R, Choi J, Bazan-Peregrino M, et al. Enhanced tumor uptake and penetration of virotherapy using polymer stealthing and focused ultrasound. J Natl Cancer Inst. 2013;7:105710–1710. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24168971.

83. Bonsted A, Engesaeter BO, Hogset A, et al. Photochemically enhanced transduction of polymer-complexed adenovirus targeted to the epidermal growth factor receptor. J Gene Med. 2006;8:286–297. Available from:
93. Kasman LM, Barua S, Lu P, et al. Polymer-enhanced adenosiviral transduction of CAR-negative bladder cancer cells. Mol Pharmacol. American Chemical Society; 2009; 6: 1612–1619. Available from: http://pubs.acs.org/doi/abs/10.1021/mp9000958
94. Jiang ZK, Koh SBS, Sato M, et al. Engineering polypeptide coatings to augment gene transduction and in vivo stability of adenosiviruses. J Control Release. 2013;166:75–85. Available from: https://www.sciencedirect.com/science/article/pii/S0168359112008334
95. Kim J, Li Y, Kim SW, et al. Therapeutic efficacy of a systemically delivered oncolytic adenosivirus - Biodegradable polymer complex. Biomaterials. 2013;34:4622–4631. Available from: https://www.sciencedirect.com/science/article/pii/S0168359112003286X
96. Choi J-W, Jung S-J, Kasala D, et al. pH-sensitive oncolytic adenosivirus hybrid targeting acidic tumor microenvironment and angiogenesis. J Control Release. 2015;205:134–143. Available from: https://www.sciencedirect.com/science/article/pii/S0168359115000061
97. Choi JW, Kim J, Bui QN, et al. Tuning surface charge and pegylation of biocompatible polymers for efficient delivery of nucleic acid or adenosiviral vector. Bioconjug Chem. 2015;26:1818–1829. Available from: http://pubs.acs.org/doi/10.1021/bc5003377
98. Fasbender A, Zabner J, Chillon M, et al. Complexes of adenosivirus with polycationic polymers and cationic lipids increase the efﬁciency of gene transfer in vitro and in vivo. J Biol Chem. 1997;272:6479–6489. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9045673
99. Kwon O-J, Kang E, Choi J-W, et al. Therapeutic targeting of chitosan–PEG–folate-complexed oncolytic adenosivirus for active and systemic cancer gene therapy. J Control Release. 2013;169:257–265. Available from: https://www.sciencedirect.com/science/article/pii/S0168359113001739
100. Kim J, Nam HY, Choi JW, et al. Efﬁcient lung orthotopic tumor-growth suppression of oncolytic adenosivirus complexed with RGD-targeted biodegradable polymer. Gene Ther. 2014;21:476–483. Available from: http://www.nature.com/articles/gt201418
101. Nosaki K, Hamada K, Takashima Y, et al. A novel, polymer-coated oncolytic measles virus overcomes immune suppression and induces robust antitumor activity. Mol Ther - Oncolytics. 2016;3:16022. Available from: http://www.nature.com/articles/mto201622
102. Rojas JJ, Sampath P, Bonilla B, et al. Manipulating TLR signaling increases the anti-tumor T cell response induced by viral cancer therapies. Cell Rep. 2016;15:264–273.
103. Grundy M, Coussios C, Carlisle R. Advances in systemic delivery of anti-cancer agents for the treatment of metastatic cancer. Expert Opin Drug Deliv. 2016;13:999–1013. Available from: http://www.tandfonline.com/doi/full/10.1517/17425247.2016.1167036
104. Romanczuk H, Galer CE, Zabner J, et al. Modification of an adenosiviral vector with biologically selected peptides: a novel strategy for gene delivery to cells of choice. Hum Gene Ther. 1999;10:2615–2626.
105. Campos SK, Barry MA. Comparison of adenovirus fiber, protein IX, and hexon capsomers as scaffolds for vector purification and cell targeting. Virology. 2006;349:453–462. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0042682206000420
106. Virgin HW. Pathogenesis of viral infection. In: Fields BN, Knipe DM, Howley PM, editors. Fields’ Virology. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins; 2007.
107. Minchinton AI, Tannock IF. Drug penetration in solid tumours. Nat Rev Cancer. 2006;6:583–592. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16682189
108. Nichols JW, Bae YH. Odyssey of a cancer nanoparticle: from injection site to site of action. Nano Today. 2012;7:606–618. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22343460
109. Maeda H, Sawa T, Konno T. Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS. J Control Release. 2001;74:47–61.
110. Baker JHE, Lindquist KE, Huxham LA, et al. Direct visualization of heterogeneous extravascular distribution of trastuzumab in human epidermal growth factor receptor type 2 overexpressing xenografts. Clin Cancer Res. 2008;14:2171–2179. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18381959
111. Tailor TD, Hanna G, Yarmolenko PS, et al. Effect of Pazopanib on tumor microenvironment and liposome delivery. Mol Cancer Ther. 2010;9:1798–1808. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20515941
112. Primeau AJ, Rendon A, Hedley D, et al. The distribution of the anticaner drug Doxorubicin in relation to blood vessels in solid tumors. Clin Cancer Res. 2005;11:8782–8788. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16361566
113. Goel S, Wong AH-K, Jain RK. Vascular normalization as a therapeutic strategy for malignant and nonmalignant disease Cold Spring Harb. Perspect Med. 2012;2:a006486. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22393352
114. Ferrara N. VEGF as a therapeutic target in cancer. Oncology. 2005;69:11–16.
115. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med. 2004;10:789–799.
116. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011;473:298–307.
117. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med. 1986;315:1650–1659.
118. Jain RK. Taming vessels to treat cancer. Sci Am. 2008;298:56–63.
119. Jain RK. Antiangiogenic therapy for cancer: current and emerging concepts. Oncol. willist. Park. 2005;19:7–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15934498
120. Kim KJ, Li B, Winer J, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature. 1993;362:841–844.
121. Gianantonio BJ, Catalano PJ, Meropol NJ, et al. Bevacizumab in combination with oxaplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol. 2007;25:1539–1544.
122. Lee CG, Heijm D, Di Tomaso E, et al. Anti-vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. Cancer Res. 2000;60:5565–5570.
123. Wildiers H, Guetens G, De Boeck G, et al. Effect of antivascular endothelial growth factor treatment on the intratumoral uptake of CPT-11. Br J Cancer. 2003;88:1979–1986.
124. Tong RT, Boucher Y, Kozin SV, et al. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res. 2004;64:3731–3736.
125. Dickson PV, Hamer JB, Sims TL, et al. Bevacizumab-induced transient remodeling of the vasculature in neuroblastoma xenografts results in improved delivery and efficacy of systemically administered chemotherapy. Clin Cancer Res. 2007;13:3942–3950.
126. Kim M, Nitschke M, Sennino B, et al. Amplification of oncolytic vaccinia virus widespread tumor cell killing by sunitinib through multiple mechanisms. Cancer Res.2018;78:922–937. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29259007
127. Kurozumi K, Hardcastle J, Thakur R, et al. Effect of Tumor Microenvironment Modulation on the Efficacy of Oncolytic Virus Therapy. JNCI J Natl Cancer Inst.2007;99:1768–1781. Available from: https://academic.oup.com/jnci/article-lookup/doi/10.1093/jnci/djm229
128. Breitbach CJ, Arulandam R, De Silva N, et al. Oncolytic vaccinia virus disrupts tumor-associated vasculature in humans. Cancer Res. 2013;73:1265–1275. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23393196
129. Hou W, Chen H, Rojas J, et al. Oncolytic vaccinia virus demonstrates antiangiogenic effects mediated by targeting of VEGF. Int J Cancer.
130. Seki T, Carroll F, Lillingworth S, et al. Tumour necrosis factor-alpha increases extravasation of virus particles into tumour tissue by activating the Rho A/Rho kinase pathway. J Control Release. 2011;156:381–389.

131. Pencavel TD, Wilkinson MJ, Mansfield DC, et al. Isolated limb perfusion with melphalan, tumour necrosis factor-alpha and oncolytic vaccinia virus improves tumour targeting and prolongs survival in a rat model of advanced extremity sarcoma. Int J Cancer. 2015;136:965–976.

132. Arulanandam R, Batenchuk C, Angarita FA, et al. VEGF-mediated induction of PRDI-BF1/blimp1 expression sensitizes tumor vasculature to oncolytic virus infection. Cancer Cell. 2015;28:210–224. Available from: https://www.sciencedirect.com/science/article/pii/S1535601815002196?via%3Dihub.

133. Miller A, Nace R, Ayala-Breton CC, et al. Perfusion pressure is a critical determinant of the intratumoral extravasation of oncolytic viruses. Mol Ther. 2016;24:306–317. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26647825.

134. Seymour LW, Fisher KD. Under pressure: elevated blood pressure enhances targeting of tumors by oncolytic viruses. Mol Ther. 2016;24:204–205. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26906615.

135. Ibsen S, Benchimol M, Simberg D, et al. Nano-biotechnology for biomedical and diagnostic research. In: Zahawy E, Ordentlich A, Yitzhaki S, et al. editors. Dordrecht: Springer Netherlands; 2012. 145–153. Available from: 10.1007/978-94-007-2555-3_14.

136. Lyon PC, Gray MD, Mannaris C, et al. Safety and feasibility of ultrasound-triggered targeted drug delivery of doxorubicin from thermo-sensitive liposomes in liver tumours (TARDOX): a single-centre, open-label, phase 1 trial. Lancet Oncol. 2018;19:1027–1039.

137. Stride EP, Coussios CC. Cavitation and contrast: the use of bubbles in ultrasound imaging and therapy. Proc Inst Mech Eng Part H J Eng Med. 2010.

138. Arvanitis CD, Bazan-Peregrino M, Rifai B, et al. Cavitation-enhanced extravasation for drug delivery. Ultrasound Med Biol. 2011;37:1838–1852.

139. Rifai B, Arvanitis CD, Bazan-Peregrino M, et al. Cavitation-enhanced delivery of macromolecules into an obstructed vessel. J Acoust Soc Am. 2010;128:310–315. Available from: http://scitation.aip.org/content/asa/journal/jasa/128/5/10.1121/1.3496388.

140. Howard CM, Forsberg F, Minimo C, et al. Ultrasound guided site specific gene delivery system using adenoviral vectors and commercial ultrasound contrast agents. J Cell Physiol. 2006;413–421.

141. Greco A, Di Benedetto A, Howard CM, et al. Eradication of therapy-resistant human prostate tumors using an ultrasound-guided site-specific cancer terminator virus delivery approach. Mol Ther. 2010;18:295–306.

142. Kwan JJ, Myers R, Coviello CM, et al. Ultrasound-propelled nanocups for drug delivery. Small. 2015;11:5305–5314.

143. Myers R, Coviello C, Erbs P, et al. Polymeric cups for cavitation-mediated delivery of oncolytic vaccinia virus. Mol Ther. 2016;24:1627–1633. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27375160.

144. Pankhurst QA, Connolly J, Jones SK, et al. Applications of magnetic nanoparticles in biomedicine. J Phys D Appl Phys. 2003;36:R167-R181.

145. Hughes C, Galea-Lauri J, Farzaneh F, et al. Streptavidin paramagnetic particles provide a choice of three affinity-based capture and magnetic concentration strategies for retroviral vectors. Mol Ther. 2001;3:623–630. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11319925.

146. Scherer F, Anton M, Schillinger U, et al. Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo. Gene Ther. 2002; 9102–109. Available from: www.nature.com/gt.

147. Mah C, Faites JTJ, Zolotukhin I, et al. Improved method of recombinant AAV2 delivery for systemic targeted gene therapy. [Cited 2018 Oct 25]. Available from: http://www.idealibrary.com.

148. Haim H, Steiner J, Panet A. Synchronized infection of cell cultures by magnetically controlled virus. J Virol. 2005;79:622–625. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15596857.

149. Pandori MW, Hobson DA, Sano T. Adenovirus-microbead conjugates possess enhanced infectivity: A new strategy for localized gene delivery. Virology. 2002;299:204–212.

150. Singh R, Kostarelos K. Designer adeno-viruses for nanomedicine and nanodiagnostics. Trends Biotechnol. 2009;27:220–229.

151. Chan L, Nesbath D, MacKey T, et al. Conjugation of lentivirus to paramagnetic particles via nonviral proteins allows efficient concentration and infection of primary acute myeloid leukemia cells. J Virol. 2005;79:13190–13194.

152. Orlando C, Castellani S, Mykhaylyk O, et al. Magnetically guided lentiviral-mediated transduction of airway epithelial cells. J Gene Med. 2010;12:747–754.

153. Kaikonen MU, Viholainen JI, Närvänen A, et al. Targeting and purification of metabolically biotinylated baculovirus. Hum Gene Ther. 2008;19:589–600.

154. Treslilwised N, Pithayanukul P, Mykhaylyk O, et al. Boosting oncolytic adenovirus potency with magnetic nanoparticles and magnetic force. Mol Pharm. 2010;7:1069–1089. Available from: http://pubs.acs.org/doi/abs/10.1021/mp100123t.

155. Zhu H, Tong S, Bao G Magnetic control of gene delivery in vivo. 2017. Available from: https://patents.google.com/patent/US20170239370A1/en.

156. Nobuto H, Sugita T, Kubo T, et al. Evaluation of systemic chemotherapy with magnetic liposomal doxorubicin and a dipole external electromagnet. Int J Cancer. 2004;109:627–635.

157. Alexiou C, Arnold W, Klein RJ, et al. Locoregional cancer treatment with magnetic drug targeting. Cancer Res. 2000;60 (23):6641–6648.

158. Wadajkar AS, Bhavsar Z, Ko CY, et al. Multifunctional particles for melanoma-targeted drug delivery. Acta Biomater. 2012;8:2996–3004.

159. Hofmann-Amtenbrink M, von Rechenberg B, Hofmann H. Superparamagnetic nanoparticles for biomedical applications. Nano Mat for Bio Appl. 2009;119–149.

160. Barnsley LC, Carugo D, Owen J, et al. Halbach arrays consisting of cubic elements optimised for high field gradients in magnetic drug targeting applications. Phys Med Biol. 2015;60:8303–8327. Available from: http://stacks.iop.org/0031-9155/60/i=21/a=8303?key=crossref.ac6a9a9ada743c0cc99f3d768f101c87.

161. Willmon C, Harrington K, Kottke T, et al. Cell carriers for oncolytic viruses: fed ex for cancer therapy. Mol Ther. 2009;17:1667–1676. Available from: https://www.sciencedirect.com/science/article/pii/S152500161632130X.

162. Adair RA, Roulstone V, Scott KJ, et al. Cell carriage, delivery, and selective replication of an oncolytic virus in tumor in patients. Sci Transl Med. 2012.

163. Berkeley RA, Steele LP, Mulder AA, et al. Antibody-neutralized reovirus is effective in oncolytic virotherapy. Cancer Immunol Res. 2018;6:1161–1173. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30209061.

164. Bell J, Roy D. Cell carriers for oncolytic viruses: current challenges and future directions. Oncolytic Virotther. 2013;47.

165. Shi Y, Hu G, Su J, et al. Mesenchymal stem cells: A new strategy for immunosuppression and tissue repair. Cell Res. 2010;20:510–518.

166. Thorne SH, Negrin RS, Contag CH. Synergistic antitumor effects of immune cell-viral biotherapy. Science. 2006;311(80):1780–1784.

167. Suárez-Álvarez B, Rodriguez RM, Calvanese V, et al. Epigenetic mechanisms regulate HMC and antigen processing molecules in human embryonic and induced pluripotent stem cells. PLoS One. 2010;5:e10192.

168. Kim J, Hall RR, Lesniak MS, et al. Stem cell-based cell carrier for targeted oncolytic virotherapy: translational opportunity and open questions. Viruses. 2015;7:6200–6217.