

# Oncolytic virotherapy in upper gastrointestinal tract cancers

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**Abstract:** Upper gastrointestinal tract malignancies are among the most challenging cancers with regard to response to treatment and prognosis. Cancers of the esophagus, stomach, pancreas, liver, and biliary tree have dismal 5-year survival, and very modest improvements in this rate have been made in recent times. Oncolytic viruses are being developed to address these malignancies, with a focus on high safety profiles and low off-target toxicities. Each viral platform has evolved to enhance oncolytic potency and the clinical response to either single-agent viral therapy or combined viral treatment with radiotherapy and chemotherapy. A panel of genomic alterations, chimeric proteins, and pseudotyped capsids are the breakthroughs for vector success. This article revisits developments for each viral platform to each tumor type, in an attempt to achieve maximum tumor selectivity. From the bench to clinical trials, the scope of this review is to highlight the beginnings of translational oncolytic virotherapy research in upper gastrointestinal tract malignancies and provide a bioengineering perspective of the most promising platforms.

**Keywords:** oncolytic viruses, hepatopancreatobiliary, gastric cancer, pancreatic cancer, liver cancer, biliary cancer

## Introduction

Gastrointestinal cancers are considered a significant challenge in public health worldwide. In the US, pancreatic adenocarcinoma and hepatocellular carcinoma (HCC) appear among the ten most deadly cancers each year.<sup>1</sup> New drugs to combat those cancers are being developed, and arrays of oncolytic viral vectors are progressing into clinical studies. Oncolytic viral therapy in upper gastrointestinal tract (UGT) malignancies represents a promising therapeutic platform. Virotherapy can allow for combination with immunotherapies or cytotoxic chemotherapies or used as a standalone platform for gene therapy in the context of cancer ultimately being a genetic disease.

Genomic profiling of UGT malignancies has yielded compendia of mutations, regions of loss of heterogeneity, gene fusions/translocations, and copy-number alterations responsible for pathogenesis, disease recurrence, and drug resistance. This review will cover the implementation of oncolytic virotherapy (OV) in esophageal, gastric, pancreatic, liver, and biliary cancers from a bedside-to-bench perspective.

## Upper gastrointestinal tract malignancies

One of the recent forays of OV in the clinic was in patients with head and neck cancers. China approved the first virus (H101) for nasopharyngeal cancers in November

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2006.<sup>2</sup> H101 is a recombinant adenovirus (Ad) very similar to ONYX15 that had been studied in the US, but efforts were unable to be sustained beyond initial clinical studies.<sup>3</sup> The approval of H101 provided a much-needed boost to the field for ongoing investigation. More recently, a herpes simplex virus (HSV)-based vector (T-Vec) garnered US Food and Drug Administration approval in 2015 for the treatment of patients with advanced melanoma.<sup>4</sup>

Pursuant to these efforts, sustained interest in the field of OV has been fostered, aided by more comprehensive understanding of the genetic underpinnings of cancers, as well as improved understanding and success of cancer immunotherapies as a mainstay of cancer treatment and a growing appreciation of the potential for evaluating OV, combined with these existing therapeutic modalities. Given the difficult prognosis of patients with advanced UGT malignancies, OV represents a promising platform for the development of novel therapeutics.

## Esophageal cancer

Esophageal cancer has an incidence of nearly 17,000 people per year in the US, and the 5-year survival is less than 19%.<sup>5</sup> Early metastasis and diagnosis at advanced clinical stage are responsible for the poor prognosis and high mortality of the disease. One of the early attempts at use of OV in esophageal cancer used herpes simplex virus type 1 (HSV1) as the viral platform.<sup>6</sup> The approach took advantage of the mucosal tropism of the strain equipped with GFP for visualization/monitoring. The cytotoxic effect of the virus was evaluated, first in vitro using BE3 cell lines, and thereafter in vivo with an intraperitoneal tumor model (Table 1). The study concluded that the viral platform could be used to mark esophageal cancer cells from a diagnostic approach at the time of endoscopy.

BE3 has served as a cell-line model for esophageal adenocarcinoma and Barrett's esophagus. Interestingly, that viral platform was derived from the HSV1 strain NV1023 that was tested in squamous-cell head and neck carcinoma and was able to spread via the lymphatic system.<sup>7</sup> The same group also tested the virus in vivo in gastric cancer models. They observed that the platform could be used to treat peritoneal carcinomatosis or demarcate it for laparoscopic intervention.<sup>8</sup>

Later, a modified Ad was developed to establish a platform that could induce cell-cycle arrest.<sup>9</sup> Increased cytotoxicity was observed against p53-mutant esophageal cancer cells. Additionally, greater oncolytic effect with reduced NFκB and enhanced apoptosis was noted.<sup>9</sup> Correspondingly, a newly modified herpes simplex virus type 2 (HSV2) was developed with oncolytic effect on esophageal cancer cell lines. The N-terminal of the *ICP10* gene of HSV2 was modified by

deleting a protein-kinase domain. This deletion increased the bystander-killing effect of the virus, enhancing apoptosis. The mutant virus was designated FusOnH2.<sup>10</sup>

A study evaluating resistant tumor cells with properties similar to the cancer stem cells was conducted using an Ad carrying an apoptotic gene, *TRAIL*, which would preferentially induce apoptosis in these resistant cells.<sup>11</sup> Further, another group evaluated the combinatorial effects of chemotherapy and oncolytic therapy with an Ad.<sup>12</sup> They used four drugs and a modified Ad5, with deletion of the 55 kDa-encoding E1B region and a part of the E3 region, generating the vector Ad-delE1B55. The four drugs tested were fluorouracil (5FU), etoposide (VP16), mitomycin C, and cisplatin (CDDP). 5FU enhanced the cell cycle at the S phase and then induced G<sub>2</sub>-M phase entry, whereas CDDP induced G<sub>1</sub> phase arrest. Ad-delE1B55 also induced the S phase and G<sub>2</sub>/M phase such that G<sub>1</sub> phase arrest by CDDP was inhibitory to Ad-mediated cell-cycle progression and as a result was unfavorable to Ad-mediated toxicities. On the other hand, the Ad-induced S phase and G<sub>2</sub>/M phase impeded CDDP-mediated cytotoxicity, and thus the cytotoxicity between the Ad and CDDP promoted cross-resistance.<sup>12</sup>

A third-generation HSV1 improved the oncolytic effect by incorporating a gene of fusogenic proteins with transgenes for gibbon-ape leukemia virus (GALV) encoded into the viral genome.<sup>13</sup> Furthermore, another group built upon their work with Ad-TRAIL-E1 toward the development of the vector Ad-hTERT-E1 (Telomelysin OBP301), under the control of hTERT.<sup>14</sup> This vector has progressed to clinical trials for esophageal cancer.

In the same year, another group evaluated the adenoviral vector H101 in esophageal carcinoma.<sup>15</sup> Following this, an enhanced antitumor effect in esophageal cancer cell lines was observed with H101 through the overexpression of coxsackie and adenovirus receptor (CAR) using trichostatin A.<sup>16</sup> Enhanced oncolytic activity was achieved through MAPK-ERK pathway induction of viral proliferation.<sup>16</sup> Recently, another study has demonstrated that trichostatin A enhances the spread and replication of adenoviral vectors in several infection-resistant cancers.<sup>17</sup>

A chimeric vector, Ad-hTERTp-E1A-HN, which uses the same adenoviral backbone as H101 coupled with the hemagglutinin-neuraminidase gene from Newcastle disease virus (NDV), has been evaluated in preclinical esophageal cancer models. Hemagglutinin-neuraminidase is a 75-kDa membrane glycoprotein that has the potential to boost innate immunity. Its neuraminidase activity allows for hydrolysis of sialic acid on the receptors of host cells. It can also induce

**Table 1** Oncolytic viral platforms for esophageal cancer

Year	Vector	Final viral construct or viral-based strain	Vector modifications	Details	Reference(s)
2003	HSV1	NV1066	Vector under CMV promoter Expressing eGFP	Could be used as a marker in endoscopy, as well as laparoscopically for carcinomatosis	6, 8
2004	Ad	AxEIAdB was used to create AxDAdB3	Deleted 55 kDa-encoding E1B region E1A gene designed to abolish binding retinoblastoma protein	Increased cytotoxicity against p53-mutant esophageal cancer cell lines, higher in vivo oncolytic effect, despite similar replication efficiency in vitro AxDAdB3 strongly reduced NFκB activity and thereby enhanced apoptosis	9
2007	HSV2	Fus-OnH2	PK domain deleted in the <i>ICP10</i> gene	Effective in tumors with resistance to apoptosis	10
2008	Ad	Ad/TRAIL-EI	Vectors under hTERT controlling E1A and TRAIL	Preferentially targeting radioresistant cells with stem-cell-like properties	11
2010	HSV1	Oncovex <sup>GALV</sup> /CD	Double deletion of the $\gamma$ 134.5 gene attenuates the virus to make it less neurotoxic Deletion of ICP47 increases antigen presentation within infected tumor cells Vector encoding a fusogenic protein with transgene-encoding GALV	Efficient infection and oncolysis in gastroesophageal cancer cells with viral platform expressing large syncytia	13
2010	Ad	Ad-delE1B55	Vectors under CMV promoter Deleted 55 kDa-encoding E1B region and a part of the E3 region	Combinatory antitumor effect depends on the chemotherapy agent	12
2011	Ad	Telomelysin OBP301	hTERT promoter Deleted 55 kDa-encoding E1B region	Intratumoral injection followed by radiation had a substantial antitumor effect	14
2014	Ad	Ad-hTERT-pE1A-HN	hTERT promoter Deleted 55 kDa-encoding E1B region Expressing HN from NDV	Suppression of tumor volume using both modes of delivery (IT and IV) Complete response to vector IT injection	18
2015	Ad	Ad SG502-TNF	SG502-TNF containing luciferase-reporter gene	SG502-TNF targeted tumor cells specifically with stable expression of TNF	19

**Abbreviations:** HSV1, herpes simplex virus type 1; HSV2, herpes simplex virus type 2; CMV, cytomegalovirus; Ad, adenovirus; PK, protein kinase; GALV, gibbon ape leukemia virus; HN, hemagglutinin–neuraminidase; IT, intratumoral; IV, intravenous; NDV, Newcastle disease virus; TNF, tumor necrosis factor.

IFN $\alpha$  and TRAIL<sup>19</sup> production in peripheral blood mononuclear cells, leading to enhancement of apoptotic pathways. The study of esophageal cancer observed cytotoxicity by an increase in reactive oxygen species. In vivo evaluation demonstrated complete responses and prolonged survival with intratumoral injection of the vector.<sup>18</sup>

A number of vectors have been entering clinical trials following these preclinical evaluations. A Phase I clinical trial for Telomelysin OBP301 is awaiting initiation ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03213054) NCT03213054). These efforts mark the transition of OV from bench to bedside in esophageal cancer. Future directions entail safety evaluation and optimal dosing of vectors, followed by combinatorial assessment with existing therapies, particularly immunotherapies.

## Gastric cancer

Gastric cancer is the fifth most common cancer in the world. It has a 5-year overall survival (OS) rate of 30.6%. Surgery

is the only curative treatment, but less than a quarter of cases are eligible.<sup>20</sup> OV for gastric cancer was initially tested in vivo in mouse models of peritoneal carcinomatosis. Initially, two oncolytic HSV vectors (G207 and NV1020) were tested. Locoregional therapy was found to be efficacious. However, systemic delivery through intravenous administration failed to diminish tumor burden.<sup>21</sup> In another study, apoptosis inhibitors were used in conjunction with viral vectors. It was found that *N*-acetyl cysteine improved killing of neighboring uninfected cells using the NV1066 platform.<sup>22</sup>

Later, the first tumor-specific promoters were tested for conditionally replicating Ads (CR-Ads) in gastric cancer.<sup>23</sup> Midkine and cyclooxygenases (Cox2M and Cox2L) promoters demonstrated high transcriptional activity in gastric cancer cells, and the Cox2CR-Ad showed a potent oncolytic effect. A fiber-modified vector, Ad5/3, also yielded robust infectivity in this study.<sup>23</sup> With its serotype 3 knob, Ad5 allows the vector to bind to a receptor different from CAR. Another study used

**Table 2** Oncolytic viral platforms for gastric cancer

Year	Vector	Final viral construct or viral-based strain	Vector modifications	Details	Reference
2002	HSV1	G207 NV1020	G207: deletion of both copies of $\gamma_1$ 34.5 genes and inactivation of <i>ICP6</i> gene NV1020: deletion of one copy of $\gamma_1$ 34.5 and intact <i>ICP6</i> gene	In vitro studies were similar, with NV1020 having better results at lower viral concentrations	21
2004	HSV1	NV1066	Early gene copies of <i>ICP0</i> and <i>ICP4</i> and the $\gamma_1$ 34.5 gene	Exposure to NAC promoted killing	22
2006	Ad	AdCEAp/Rep	CEA promoter (conditionally replicating)	Cytotoxicity against CEA-producing cells was dose-dependent and different from CEA-negative cells	25
2009	Ad	AdE2F-p16	p16-expression cassette was inserted upstream of the E2F promoter Data were normalized with those of CMV promoter	Tumor-inhibition rate in xenografts was 59%	26
2009	EVI	Farouk virus	No alterations were made	Specific targeting of $\alpha 2\beta 1$	27
2009	Ad	Ad5/35	Fiber shaft and knob from Ad35 into Ad5 E1B deleted SG235-TRAIL	More efficient cell entry	45
2010	NDV	NDV(F3aa)-GFP	Cleavage site of the F protein was modified with three amino-acid changes, making it more fusogenic Expressing GFP	Treated mice exhibited a significant reduction in tumor development and prolonged survival	28
2010	Reovirus	ReoT3D	No alterations were made	Diminished ascites volume and tumor burden	30
2012	HSV1	HSV (T-TSPI)	Expressing TSPI	Arming the virus attenuated its replication in some cell lines, but cytotoxicity was not affected Enhanced inhibition of angiogenesis was observed	31
2012	Ad	Ad-hTERT-E1A-apoptin	Ad under hTERT promoter with E1A and apoptin	Ad-hTERT-E1A-apoptin resulted in complete response to treatment	32
2013	Ad	Telomelysin OBP301	hTERT promoter Deleted 55 kDa-encoding E1B region	OBP-301 induced cell-cycle mobilization from $G_0$ - $G_1$ to $S/G_2/M$ phases and subsequent cell death in quiescent CD133 <sup>+</sup> cancer stem-like cells by mobilizing cell-cycle-related proteins	33
2013	Ad	E1B55KD (Add11520) Endostatin Ad-Endo	Deleted 55 kDa-encoding E1B region Expressing endostatin	Selective replication and expression of endostatin in gastric cancer cells achieved synergistic effect	37
2013	Ad	AdSurp-Hsp70	Under survivin promoter Expressing chaperone Hsp-70	Selective replication in surviving positive tumor cells	38
2014	Vaccinia	GLVI h153	Expressing Ruc-GFP fusion protein Encoding rTFR, hNIS Encoding thymidine kinase and hemagglutinin	Tumor regression over a 4-week period, with one animal with total regression	39
2015	NDV	La Sota strain NDV-rL-RVG	Expressing the rabies virus glycoprotein (rL-RVG)	Induced significant autophagy	40
2017	Ad	Ad/TRAIL-EI	TRAIL gene expression	Vector delivery through peritoneal injection was effective	42
2017	NDV	NDV-D90	Wild-type NDV isolated in China; no alterations were made	Reduced gastric cancer growth by inducing apoptosis and impairing gastric cancer vascularization	43

**Abbreviations:** HSV, herpes simplex virus; HSV1, herpes simplex virus type 1; NAC, N-acetylcysteine; CMV, cytomegalovirus; Ad, adenovirus; CEA, carcinoembryonic antigen; EV, echovirus; NDV, Newcastle disease virus; Ruc, *Renilla Aequorea* luciferase; rTFR, reverse-inserted human transferrin receptor; hNIS, human sodium-iodide symporter.

this Ad5/3 capsid in an Ad5LucRGD vector. Incorporation of peptides Arg–Gly–Asp (RGD) in the fiber knob allowed the virus to utilize  $\alpha_v\beta$ -class integrins for binding and internalization. These integrins are highly expressed in gastric cancers. The vector Ad5LucRGD also had polylysine motifs – pK7 and pK21 – that bound to heparan sulfates, which are overexpressed in gastric cancers. The receptor for Ad3 is believed to be CD46, which is also highly expressed in advanced gastric cancers. From this perspective, the chimeric capsid considerably improved viral infectivity.<sup>24</sup>

In another study, a CEA promoter was introduced into an adenoviral vector to study conditional vector replication in CEA-expressing gastric carcinoma cells, paving the path for a CEA-promoter-driven CR-Ad in gastric cancers.<sup>25</sup> Further, a viral platform targeting the cyclin D–CDK–p16–Rb–E2F pathway was created based on an Ad by inserting a p16-expression cassette upstream of the E2F promoter, thereby controlling E1A with the E2F promoter. This AdE2F-p16 replicated preferentially in tumor cells with the desired specificity, while sparing normal cells. Luciferase assays demonstrated that the promoter conferred vector selectivity in cancer cells compared to normal cells (Table 2).<sup>26</sup> In a contemporaneous evaluation, echovirus 1 was evaluated for peritoneal dissemination in gastric cancer models by vector dissemination through the use of  $\alpha_2\beta_1$  integrins.<sup>27</sup>

A subsequent study evaluated NDV as an oncolytic in gastric cancer. A bioengineering modification improved its fusogenic proteins by deleting three amino acids through reverse genetics. The new virus, NDV(F3aa), was able to reduce the tumor burden in animal models of gastric cancer.<sup>28</sup> Reovirus has also been studied in gastric cancer. It has a double-stranded RNA genome, and its oncolytic action depends on the presence of activated Ras-signaling pathways. This activation could be via direct Ras mutations, such as K-Ras or N-Ras, or downstream of other pathway activators, such as EGFR, Her2 (Neu/ErbB2), or SOS.<sup>29</sup> These attributes make reovirus a desired OV candidate for targeting cancers that have Ras activity. In 2010, a study evaluating reovirus in Ras-activated gastric cancer models showed encouraging efficacy.<sup>30</sup>

Gene therapy-based approaches to modulate the tumor microenvironment have also been pursued using viral vectors in gastric cancer. An HSV armed with thrombospondin 1 was evaluated in gastric cancer.<sup>31</sup> Another study used apoptin to induce apoptosis specifically in tumor cells. Apoptin is a p53-independent, Bcl2-insensitive apoptotic protein that can induce programmed cell death only in cancer cells. An Ad under the hTERT promoter was utilized to carry the apoptin gene (Ad-hTERT-E1A-apoptin). In vitro and in vivo evalua-

tions yielded promising efficacy, including a number of complete tumor regressions.<sup>32</sup> The apoptin gene is derived from the chicken anemia virus, and it does not need a functional p53 pathway nor is it hindered by Bcl2 blockage of apoptosis.

Furthermore, another group evaluated the Ad OBP301 to target quiescent stem-like cells.<sup>33</sup> These types of cells are largely resistant to conventional cytotoxic therapies as long as they are in a dormant phase. Mobilizing these quiescent cells into the cell cycle can reenact a response to treatment. This study demonstrated that cell-cycle mobilization and S/G<sub>2</sub>/M phase trapping could be induced by adenoviral infection. This was felt to be a significant accomplishment in solid-tumor OV, given that mobilization of quiescent cells into the cell cycle had only been previously achieved in leukemia.<sup>34,35</sup>

Another study described a viral gene-therapy approach to create an Ad with endostatin.<sup>36</sup> Endostatin is a 20 kDa C-terminal fragment of collagen XVIII known to be a potent inhibitor of angiogenesis.<sup>37</sup> Synergistic effects of oncolysis and endostatin expression were achieved allowing for enhancement of virotherapy efficacy. In the same realm, another study evaluated an adenoviral platform with a survivin promoter and a chaperone gene – *HSP70*. The resultant vector (AdSurp-Hsp70) replicated selectively in survivin-positive gastric cancer cells and inhibited tumor growth in both immunodeficient and immunocompetent mice.<sup>38</sup>

Conversely, a vaccinia virus with visible markers has been used for deep-tissue imaging in gastric cancer.<sup>39</sup> The markers used were GFP and human NIS, which can be readily imaged with <sup>99m</sup>Tc scintigraphy and <sup>124</sup>I positron-emission tomography. These types of approaches allow for noninvasive vector monitoring. Recently, a number of studies have evaluated chimeric vectors. An example of such a vector is the NDV La Sota strain expressing the rabies virus glycoprotein. This chimeric vector was found to have increased oncolytic efficacy in gastric cancer cell lines.<sup>40</sup>

Besides vector development, delivery strategies have also been shown to have an impact on antitumor efficacy.<sup>41</sup> An intraperitoneally delivered adenoviral vector exhibited efficacy in diminishing peritoneal carcinomatosis in a gastric cancer model.<sup>42</sup> There has been considerable interest in evaluating wild-type virus strains known to have oncolytic properties. A wild-type NDV strain isolated in China named NDVD90 has shown promising preclinical efficacy in gastric cancer, selectively killing gastric cancer cells with no appreciable effect on normal cells.<sup>43</sup> Vaccinia virus vectors have also garnered interest toward evaluation in gastric cancer. A clinical study evaluating a vaccinia viral oncolytic vector (GL-ONC1) was completed in Germany in gastric cancer

patients with peritoneal carcinomatosis ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01443260) NCT01443260).<sup>44</sup> Recently, synergistic cytotoxicity has been reported with concomitant OV and chemotherapy.<sup>45</sup>

## Pancreatic cancer

Pancreatic cancer is one the most challenging malignancies, with a 5-year OS of only 8.2%.<sup>46</sup> Unfortunately, this rate has not changed over the last 20 years, despite some recent modest advances in systemic therapy with FOLFIRINOX and gemcitabine–*nab*-paclitaxel. As such, novel therapeutic approaches are in imminent need and oncolytic viruses represent a promising treatment approach in this regard. A

number of clinical studies evaluating oncolytic viruses are ongoing (Table 3). Translational research is the main focus of these vectors being evaluated in pancreatic cancer.

Initially, such vectors as ONYX015 (Ad with E1B 55 kDa deletion) developed to treat other malignancies were evaluated in pancreatic tumor cancer cells. Findings were similar to other tumors, with the vector replicating in TP53-deficient cells.<sup>47</sup> Since then, the field has quickly evolved to address specific challenges related to pancreatic tumors. From this perspective, research efforts have focused on overcoming 5FU resistance and using a gene-therapy approach: this effort has yielded the Ad AxE1AdB-UPRT. This Ad

**Table 3** Current clinical trials using oncolytic viruses for pancreatic cancer

Vector	Viral construct and/or alteration	Phase	Vector intervention	Associated treatment	Trial location	ClinicalTrials.gov identifier
Ad	Ad5-yCD/mutTKSR39rep-hIL12 Oncolytic Ad expressing two suicide genes and human IL12	I	Single IT injection	Followed by 7 days of 5FU 14 days after 5FU chemotherapy at clinician discretion	USA	NCT03281382
HSV1	HF10, a spontaneous HSV mutant	I	IT injection	Gemcitabine (once a week, 3 weeks, followed by 1 week of rest) <i>nab</i> -paclitaxel weekly for 3 weeks followed by 1 week of rest	Japan	NCT03252808
Ad	LOAd703 Oncolytic Ad serotype 5/35 encoding TMZ-CD40L and 4-1BBL Expresses transgenes Trimerized membrane-bound isoleucine zipper (TMZ) TMZ-CD40L and 41BBL	I/II	Image-guided IT injection	Standard-of-care chemotherapy If standard of care not an option, then immunoconditioning	Sweden	NCT03225989
Ad	LOAd703	I/II	Phase I: image-guided IT injection at the same time as chemotherapy Phase II: treatment at maximum tolerated dose as defined in Phase I	Standard-of-care chemotherapy If standard of care not an option, then immunoconditioning	USA	NCT02705196
Reovirus	Serotype 3 with no alterations	I	Intravenous infusion administered on days 1 and 2 of a 21-day cycle	In combination with one of three chemotherapy drugs (gemcitabine or irinotecan or leucovorin), followed by 5FU	USA	NCT02620423
Ad	VCN01 expressing PH20 hyaluronidase	I	Intravenous dose escalation, single agent, and combination	With or without gemcitabine/ <i>nab</i> -paclitaxel	Spain	NCT02045602
Ad	VCN01 Expressing PH20 hyaluronidase	I	Three IT administrations of VCN01 Ad every 28 days in combination with <i>nab</i> -paclitaxel and gemcitabine	Gemcitabine plus <i>nab</i> -paclitaxel	Spain	NCT02045589
PV	Parvovirus HI	I/II	Intravenous dose on 4 consecutive days and intrametastatic dose 6–13 days thereafter	None	Germany	NCT02653313

**Abbreviations:** Ad, adenovirus; IT, intratumoral; HSV, herpes simplex virus; HSV1, herpes simplex virus type 1; FU, fluorouracil.

vector is capable of expressing UPRT, which can overcome 5FU resistance.<sup>47</sup> Given that host antiviral immunity can be an impediment to efficacy, evaluation was undertaken in immunocompetent models. Other studies have utilized immunosuppression with cyclophosphamide accompanying vector delivery in immunocompetent models, with encouraging results.<sup>48</sup>

In 2007, an Ad carrying suicide genes was successfully tested in preclinical models of pancreatic cancer. Ad5-yCD/mutTKSR39rep-ADP is an Ad5 vector carrying Ad cytosine deaminase and HSV thymidine kinase (TK<sub>SR39</sub>). This vector augmented the effectiveness of pancreatic radiotherapy without resulting in excessive toxicity.<sup>49</sup> Pursuant to this, other adenoviral vectors carrying TK genes in combination with ganciclovir were tested using an intraductal delivery approach, resulting in improved survival *in vivo*.<sup>50</sup>

The La Sota strain of NDV has been tested in pancreatic cancer models, with favorable efficacy. Pancreatic tumor-cell lines were 700 times more sensitive to cytotoxicity from the NDV La Sota strain in *in vivo* pancreatic cancer models than normal pancreatic cells.<sup>51</sup> Likewise, reovirus has been evaluated in pancreatic cancer. Given that it replicates only in cells that express an active Ras pathway and the nearly ubiquitous nature of Ras activation in pancreatic cancer, this virus became particularly interesting in this disease.<sup>52</sup> In one study evaluating reovirus in pancreatic cancer, the vector proliferated to yield a considerable amount of viral particles and ubiquitinated proteins in the endoplasmic reticulum, causing apoptosis mediated by endoplasmic reticulum stress *in vitro* and *in vivo*.<sup>52</sup>

Human and avian influenza A viruses (IAVs) have also been evaluated in pancreatic cancer models.<sup>53</sup> The appeal of these platforms was based on the natural tropism of IAV for pancreatic cells. The study confirmed the expression of  $\alpha_{2,3}$ - and  $\alpha_{2,6}$ -linked polysaccharide receptors, respectively, for avian and human IAV in pancreatic ductal adenocarcinoma (PDAC) cell lines. Correspondingly, IAVs did not infect normal pancreatic cells. Tropism and selectivity were observed, with promising antitumor efficacy both *in vitro* and *in vivo*.<sup>53</sup>

Tumor selectivity was a critical impetus for a study in pancreatic cancer that involved targeting MMP with an Ad bioengineered with a TAT-like peptide and linked to a blocking domain by an MMP-cleavable sequence and achieving MMP-dependent transduction. This viral platform, AdTAT-MMP, was shown to reduce local and distant metastases, with no significant toxicity in *in vivo* pancreatic cancer models.<sup>54</sup>

From the perspective of tumor selectivity, one research group identified a pancreatic cancer-targeting ligand

(SYENFSA) that would boost the specificity of an Ad under a survivin promoter, achieving higher transduction efficiency. Ad Sur-SYE resulted in potent oncolysis in PDAC<sup>55</sup> and superior efficiency when compared to the non-targeting virus AdSur for neuroendocrine pancreatic tumors when administered intratumorally.<sup>56</sup>

In 2015, the oncolytic Ad VCN01 was also tested *in vitro* and *in vivo* for PDAC. VCN01 is characterized by the expression of hyaluronidase and the RGD shaft-retargeting ligand. As such, VCN01 employs a strategy whereby the tumor microenvironment can be modulated using a viral vector. Encouraging safety and efficacy have been noted with VCN01 in preclinical studies, paving its way toward clinical development.<sup>57</sup>

Second-generation HSV1 vectors have been evaluated in pancreatic cancer. Using recombinant HSV1, a novel viral platform was created with ICP6-defective expression and HSV1  $\gamma$ 134.5 gene expression, regulated by the cellular B-Myb promoter and giving rise to the viral vector Myb34.5.<sup>58</sup> Tumor progression was inhibited with intratumoral injection of this virus alone, and tumor regression was achieved when virotherapy was combined with standard chemotherapeutic agents. In light of the promising preclinical data generated in these and other evaluations, a number of oncolytic vectors have progressed into clinical studies (Table 3).

## Liver and biliary cancers

Liver and intrahepatic bile duct cancer affect around 40,000 people per year in the US, with nearly 29,000 deaths each year and a dismal 5-year OS of only 17.6%.<sup>59</sup> Toxicity to neighboring hepatocytes is a significant concern, and vectors have been developed to increase tumor specificity and decrease off-target toxicity. In this section, we review both DNA and RNA viruses used as oncolytic vectors for liver and biliary cancers and acknowledge current oncolytic clinical trials addressing those tumors in Table 4.

## DNA viruses: adenovirus, herpes viruses, and vaccinia

The first HCC-specific Ad (CV890) was reported using transcriptional regulatory elements of AFP as replication drivers for Ad5. The vector has E1A and E1B genes under the control of a bicistronic AFP-E1A-IRES-E1B cassette.<sup>60</sup> *In vivo* results demonstrated tumor-volume reduction below baseline 4 weeks after a single viral dose. When combined with doxorubicin, there was synergistic antitumor efficacy, with complete remission after a single instance of combination therapy.<sup>60</sup> Using the ONYX015 adenoviral vector

**Table 4** Clinical trials with oncolytic viruses for hepatobiliary tumors

Vector	Vector details	Phase	Vector intervention	Associated treatment	Trial location	ClinicalTrials. Gov identifier
Vaccinia virus	Recombinant Wyeth strain Pexa-Vec (JX594)	I/II	Three biweekly IT injections	Nivolumab intravenously every 2 weeks	France	NCT03071094
Vaccinia virus	Recombinant Wyeth strain Pexa-Vec (JX594)	III	Three biweekly IT injections	Sorafenib twice daily	Multicentric (98 locations, up to 600 patients)	NCT02562755
Adenovirus	Telomelysin OBP301	I/II	Dose escalation	None	Korea, Taiwan	NCT02293850
Adenovirus	Recombinant Ad5	III	Followed by TACE	TACE with chemotherapy drugs vs TACE with virus	China	NCT01869088
Vaccinia	JX594 recombinant vaccinia GM-CSF	II	Five intravenous weekly infusions on days 1, 8, 15, 22, and 29	None	USA, Spain, South Korea	NCT01636284
Vesicular stomatitis virus	Recombinant vesicular stomatitis virus-expressing IFN $\beta$	I	Single-dose IT injection	None	USA	NCT01628640
Vaccinia	JX594 recombinant vaccinia GM-CSF	II	Treatments on days 1, 8, 22, week 6, week 12, and week 18	Best supportive care	Multicenter	NCT01387555
Vaccinia	Thymidine kinase-deleted vaccinia virus plus GM-CSF	II	IT dose on days 1, 15, 29	None	Canada, South Korea, USA	NCT00554372
Adenovirus	Gene therapy Ad5 encoding <i>TP53</i> gene	I	Treatment occurs every 28 days for up to six courses	None	USA	NCT00003147

**Abbreviations:** Pexa-Vec, pexastimogene devacirepvec; IT, intratumoral; Ad, adenovirus; TACE, transarterial chemoembolization.

platform, a murine endostatin-carrying vector was evaluated in HCC. A favorable safety profile and antitumor efficacy were both exhibited.<sup>61</sup>

A dual-promoter approach has been evaluated as a strategy to enhance tumor specificity and delivery of gene therapy in HCC. A conditionally replicative gene–viral vector system (CNHK500-p53) employed insertion of a *TP53* gene-expressing cassette into the viral genome coupled with an hTERT promoter to drive the E1A adenoviral gene and a hypoxia-response-element promoter to drive the adenoviral E1B gene, with the objective of producing p53 protein in hypoxic drive, telomerase-positive HCC cells.<sup>62</sup> In vitro studies demonstrated that p53 expression enhanced virus oncolytic effect.<sup>62</sup> Besides *TP53*, another tumor suppressor gene, *TSLC1*, was evaluated using an Ad platform (SD55-TSLC1).<sup>63</sup> In order to increase oncolytic efficacy, another strategy has been to add lethal genes from mitochondria to be expressed by the vector in tumor cells. In 2007, a vector encoding the Smac protein was developed (ZD55-Smac). ZD55-Smac was a superior oncolytic vector but cytotoxicity towards normal cells was a concern.<sup>64</sup>

A number of enhancements to increase safety have been made beyond the initial vector approaches. Tumor-specific promoters have aided with vector selectivity toward tumors

in HCC. AFP and transthyretin<sup>65</sup> promoters have been deployed in adenoviral vectors. Similarly, an AFP-regulated ribonucleotide reductase was instituted in a HSV vector, resulting in increased oncolytic effect.<sup>66</sup> A number of promoters have been evaluated in HCC in HSV vectors, which include ANGPTL3, CYP2C8, vitronectin, ADH6, APOB, FBC, ITIH3, and ITIH1.<sup>67</sup> Recently, the promoter for a Golgi protein, GOLPH2 (also called GP73), was successfully instituted as an HCC-specific Ad promoter.<sup>68</sup>

Hybrid promoters are also being used. One such example is HRE-AFP, used in a construct with the bee-venom toxic peptide melittin. Melittin can induce HCC apoptosis, and the vector QG511-HA-melittin exerted an inhibitory effect in HCC and was described as triple-targeting mechanism addressing AFP-positive cells, cells in a hypoxic environment, and cells with p53 deficiency.<sup>69</sup> Furthermore, the use of microRNA (Let7) to control replication was introduced to diminish toxicity.<sup>70</sup> A study utilizing the micro-RNA miR34a for vector specificity allowed for delivery of IL24.<sup>71</sup> Recently, an Ad-expressing lncRNA that can competitively bind oncogenic miRNAs has been shown to achieve promising antitumor efficacy.<sup>72</sup>

A wide range of genes that can elicit oncolytic effects has been evaluated to date. *SOCS3* can downregulate cyclin D1



and antiapoptotic proteins and enhance antitumor effects.<sup>73</sup> Similarly, *SOCS1*, a negative regulator of STAT3, can inhibit STAT3 phosphorylation and downregulate survivin, cyclin D1, Bcl-xL and C-myc.<sup>74</sup> Another strategy to enhance potency relies on oxygen-dependent degradation domain-regulated vectors that attract NK92 cells into hypoxic tumor microenvironments in animal models.<sup>75</sup> Similarly, a construct using manganese superoxide dismutase (AD55-Mn-SOD) has been shown to suppress HCC growth effectively in xenografted nude mice.<sup>76</sup>

Despite Ad and cisplatin being described as having competitive modes of action, one study has found that cisplatin can be used in combination with Ad. Growth inhibition of HCC by XAF1 in an adenoviral platform (ZD55-XAF1) was pursued in these efforts.<sup>77</sup> XAF1 is a counterregulator of the IAP protein family. With this vector, reduced cisplatin doses were possible and tumor-cell apoptosis enhanced via the activation of the caspase 9–PARP pathway.<sup>77</sup>

Another DNA virus, the vaccinia-based construct JX594, has been shown to be a promising candidate for viroimmunotherapy. Immunostimulation was augmented by GM-CSF transgene expression and evaluated in three patients with hepatitis B-related HCC. In this study, virotherapy was found to induce antivascular cytokines and was associated with tumor vascular shutdown. Interestingly, it also appeared to suppress hepatitis B replication.<sup>78</sup> Correspondingly, another vaccinia strain, GLV1h68, was evaluated in vitro and exhibited cytotoxicity in both sorafenib-sensitive and -resistant HCC cell lines.<sup>79</sup> Preliminary consideration for an immunomediated mechanism of action has been proposed, given observation of tumor response in distant, noninjected tumors in an ongoing clinical study with JX594.<sup>80</sup>

## RNA viruses: vesicular stomatitis virus, measles virus, Newcastle disease virus, and retroviruses

Vesicular stomatitis virus (VSV) is a negative-strand RNA virus that been tested in vitro and in vivo in immunocompetent mice, with evidence of tumor shrinkage and minimal toxicity.<sup>81</sup> Subsequently, other recombinant forms of VSV, such as rVSV- $\beta$ -galactosidase, which expresses  $\beta$ -galactosidase to facilitate X-galactosidase staining of tumors, were tested in HCC. rVSV- $\beta$ -gal was enabled with tracking capabilities to evaluate multifocal viral spread after arterial delivery of the vector.<sup>82</sup>

Further interest in VSV with chimeric genes to enhance its antitumor effect led to the development of a fusogenic VSV expressing the fusion protein of NDV (rVSV-NDV/F). This

new vector was superior to wild-type VSV, with enhanced oncolysis due to syncytia formation and resulted in prolonged survival of rats in vivo.<sup>83</sup> Continued VSV genome engineering yielded improved vector safety using an M $\Delta$ 51 deletion in the viral genome. Pursuant to this, the expression of M3, a broad-spectrum and high-affinity chemokine-binding protein from murine  $\gamma$ HSV68, enhanced oncolytic potency, resulting in the vector rVSV(M $\Delta$ 51)-M3.<sup>84</sup> VSV-hIFN $\beta$  was also built around the cornerstone of achieving enhanced safety by using human IFN $\beta$  to protect neighboring normal cells from oncolytic damage.<sup>85</sup> This vector is currently undergoing clinical trials.

Other RNA viruses have also been tested in HCC. In 2006, the measles virus Edmonston strain (MV-Edm) entered the scene, due to the establishment of overexpression of CD46 receptors in HCC. CD46 is one of the entry receptors used by MV allowing for cellular entry and syncytia formation. Engineered MV-Edm vectors expressing CEA or human NIS were evaluated in HCC, and demonstrated promising oncolytic potential.<sup>86</sup> MV has also been bioengineered with the suicide gene super-cytosine deaminase, which promotes apoptosis-like death that is not dependent on an intact apoptotic pathway.<sup>87</sup>

NDV is another RNA virus that has been tested in HCC models. An NDV vector harboring an L289A mutation within the F gene resulted in enhanced fusion and cytotoxicity of HCC cells in vitro compared to an rNDV/F3aa control virus.<sup>88</sup> Experiments with RNA retroviruses have demonstrated oncolytic effect in HCC. Vectors derived from the murine leukemia virus-based replicating retrovirus vector (RRV) and GALV have been evaluated in HCC. GALV RRV-mediated suicide gene therapy efficiently suppressed HCC tumor growth in vivo, and no detectable RRV signals were observed outside the tumor.<sup>89</sup>

Cholangiocarcinoma receptors have exhibited a wider level of heterogeneity with regard to receptors for viral entry. One study addressed this issue by modifying the vector with ligands that would better enable integrin-dependent infection. An RGD-fiber modification was used to improve the infectivity and subsequent efficacy of adenoviral vectors in biliary cancers.<sup>90</sup> Biliary cancers remain somewhat understudied with regard to OV. Additional studies are needed in this regard to better inform OV approaches in biliary cancers.

## Future directions

Despite the continuous evolution of vectors, particularly at the genomic level, and the increasing tumor specificity achieved, it is clear that the overall efficacy of OV therapy needs to be improved. One aspect of significant concern is

the delivery method, which needs to be designed in a way to enhance viral bioavailability and viral dissemination throughout the tumor. An excellent delivery tool would provide a shield to immunoneutralization and would also increase virus bystander-killing potency by enhancing tumor-cell infection. Another approach to improve oncolytic efficacy is to enhance the replication capacity of tumor-selective viral strains to achieve better tumor penetration. The challenges observed to date show that the more bioengineering modifications a virus is subjected to, the less potent it becomes in relation to its wild type. There is no genomic manipulation thus far that has been able to increase potency. As such, after safety and tumor specificity have been developed, it is about time for future work to show an increase in killing power of safe vectors.

## Conclusion

Oncolytic vectors are very diverse, and their development has evolved to establish tumor selectivity and safety. For cancers with dismal OS rates, virotherapy represents a promising therapeutic platform for further evaluation. A broader array of clinical studies evaluating OV is anticipated in the years to come. In particular, combinations of virotherapies with immunotherapies, such as immun checkpoint inhibitors and standard cytotoxic chemotherapies, will be of particular interest. More tailored, precision-medicine-informed approaches will likely also constitute an area of future exploration. A number of challenges are yet to be addressed to allow for the realization of the full potential of OV. These include drug delivery of vectors to tumors with sequestration in normal tissues/organs, ability to overcome/avoid host immunoresponse to allow for repeated dosing, and ability to enable systemic administration of vectors to permit broader adaptation of these therapies. The framework to address these challenges and explore the myriad available opportunities has been laid, and as such, a brave new era awaits this burgeoning field.

## Disclosure

The authors report no conflicts of interest in this work.

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