

# Oncolytic virotherapy for head and neck cancer: current research and future developments

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**Abstract:** Head and neck cancer (HNC) is the sixth most common malignancy worldwide. Despite recent advancements in surgical, chemotherapy, and radiation treatments, HNC remains a highly morbid and fatal disease. Unlike many other cancers, local control rather than systemic control is important for HNC survival. Therefore, novel local therapy in addition to systemic therapy is urgently needed. Oncolytic virotherapy holds promise in this regard as viruses can be injected intratumorally as well as intravenously with excellent safety profiles. This review will discuss the recent advancements in oncolytic virotherapy, highlighting some of the most promising candidates and modifications to date.

**Keywords:** head and neck cancer, virotherapy, oncolytic viruses

## Introduction

Head and neck cancer (HNC) remains a global health concern accounting for 650,000 new cases each year, resulting in 350,000 deaths worldwide. The identification of human papillomavirus (HPV) infection as a favorable prognostic factor has fueled clinical studies investigating deintensification strategies in this HPV-positive disease subset. However, the overall survival of HPV-negative tumors remains poor despite intensive therapy. Early stage localized tumors are often cured with a single modality, including surgical resection or radiation therapy; however, locally advanced HNC often requires multimodality treatment. Despite primary intensive treatment of locally advanced disease, relapse rates at 2 years remain at 30%–50%,<sup>1–3</sup> resulting in 5-year survival of less than 10% in the relapsed and metastatic group.<sup>4</sup> Even though the addition of targeted agents, such as the epidermal growth factor receptor (EGFR) inhibitor, cetuximab, has resulted in incremental rise in overall survival both in locally advanced and metastatic disease, the overall impact has been minimal.<sup>4,5</sup> Novel therapeutic strategies are desperately required to further advance the success in HNC treatment. Several exciting areas of investigation are ongoing, including other molecular-targeted therapy, immunotherapy, gene therapy, as well as oncolytic virotherapy, as means of improving treatment outcomes and reducing morbidity in HNC treatment. Here, we review the background, feasibility, and potential role for oncolytic virotherapy in the treatment in HNC.

## Oncolytic virotherapy

Oncolytic virotherapy is a promising approach as a cancer therapeutic, especially in its potential for local tumor control and low morbidity. Oncolytic viruses have the ability

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to selectively replicate in tumor cells via deletion of critical virulence genes that are required for replication in the normal host. Several oncolytic viruses have been tested both preclinically and in clinical trials (Tables 1 and 2), demonstrating safety and, in some instances, activity in human tumors. HNC allows for a unique opportunity for the application of these viruses as locoregional control is often most critical in this disease. Here, we will review the most common oncolytic viruses and their potential use as therapy in HNC.

## Oncolytic adenovirus

One of the first engineered replication selective viruses to be tested in humans in clinical trials are the adenoviruses. The adenoviruses are naturally cytolytic, nonenveloped, double-stranded DNA viruses. Oncolytic adenoviruses contain modifications conferring tumor-selective replication. Modifications include deletion of the p300/CBP-binding or the pRB-binding region of E1A (resulting in selective killing of tumor cells with defects in the Rb pathway), or deletion/mutation of the E1B-55 K gene resulting in the loss of p53 degradation and late viral RNA export.<sup>6-8</sup>

Several oncolytic adenoviruses have been tested in preclinical models, including those with viral modifications to enhance tumor cell killing and delivery, as well as in combination with conventional treatments. Ad5/3-delta24, a capsid-modified oncolytic adenovirus, was synergistic with conventional chemotherapy, including cisplatin and 5-fluorouracil (5-FU), as well as with radiation in a laryngeal squamous cell carcinoma model *in vivo*.<sup>9</sup> Interestingly, the addition of cetuximab to chemotherapy and radiation resulted in complete prevention of tumor relapse in xenografts compared to cetuximab alone. In order to limit toxicity of 5-FU selectively to tumor cells, oncolytic adenoviruses were genetically engineered to express an FCU1 protein, which allows for conversion of 5-fluorocytosine to the active drug 5-fluorouridine.<sup>10</sup> Despite decreased viral DNA replication in the tumors, there continued to be increased levels of apoptosis providing evidence for conversion to 5-FU selectively in the tumor.

Due to upregulation of CD46 receptor on many tumors, the Ad $\Delta$ 1520 (ONYX-015, Onyx Pharmaceuticals, California, USA) virus was genetically modified to create an Ad5/35 chimeric virus by substituting the Ad5 fiber or penton for the Ad35 fiber, which recognizes CD46, to enhance potency.<sup>11</sup> This virus has shown good tumor selectivity and safety, but durable objective responses with this virus as a single agent have been limited, and this could be partly due to the loss of other essential functions of the deleted genes.<sup>12</sup> However, the Ad5/35 chimeric oncolytic adenovirus

in combination with cisplatin demonstrated delayed tumor progression compared to Ad5/35 alone or the combination of Ad5 and cisplatin.<sup>13</sup> Similar results were also seen when Ad5/35 was combined with radiation, suggesting that combining this virus with current treatment may be an effective therapeutic strategy.

In an immunocompetent Syrian hamster animal model of HNC, Vijayalingam et al<sup>14</sup> evaluated the effect of two apoptogenic human adenovirus (HAdV) vectors. They established two cell lines of hamster cheek pouch squamous cell carcinomas, induced by treatment with 9,10-dimethyl-1,2-benzanthracene. These cell lines, when infected with HAdV5 mutants lp11w and lp11w/D55 K (which are defective in the expression of either E1B-19 K alone or both E1B-19 K and E1B-55 K proteins), exhibited enhanced apoptotic and cytotoxic responses. Intratumoral administration of HAdV5-E1B mutants efficiently suppressed the growth of tumors, suggesting the potential of these viruses in HNC treatment. These tumors also exhibited increased caspase-3 levels, suggesting that viral-induced apoptosis may contribute to tumor growth suppression.

In a nasopharyngeal carcinoma (NPC) model, the replication-deficient adenovirus encoding human endostatin (Ad-Endo) demonstrated to have promising antiangiogenic and antitumoral effects. In addition, the combination of H101 (an E1B-deleted adenovirus) and Ad-Endo significantly inhibited CNE-2 xenografts growth through increased endostatin expression and Ad replication.<sup>15</sup> Similar results were obtained using the E1B-55 K-attenuated adenovirus,  $\Delta$ 11520 in neural progenitor cells (NPCs) and xenografts in nude mice. Importantly, the antitumor activity of  $\Delta$ 11520 was augmented by the addition of cisplatin both *in vitro* and *in vivo*, showing that  $\Delta$ 11520 and cisplatin have a synergistic anti-NPC effect through oncolysis and the induction of apoptosis.

Lee et al<sup>16</sup> described a genetically modified adenovirus (Ad-DE1B19-RLX), in which the E1B19 region was deleted and relaxin (RLX) was inserted in the site of the E3 region to express RLX, affecting collagen metabolism and improving intratumoral spread of the virus. In animal experiments, Ad-DE1B19-RLX significantly reduced the size of oral squamous cell carcinoma (OSCC) tumors. There was no significant difference between radiosensitive and radioresistant OSCC.<sup>16</sup> Additionally, the OBP-301, a telomerase-specific replication-selective adenovirus, was also able to overcome radioresistance of HNC in an orthotopic nude mouse model.<sup>17</sup> Infection with OBP-301 was found to enhance the antitumor efficacy of radiation, both *in vitro* and *in vivo*, by inhibiting MRN complex expression and increasing apoptosis induction. Additionally, OBP-301 either alone or in combination with

Table 1 Summary of oncolytic viruses in clinical studies

Virus	Mechanism of selectivity/action	Method of administration	Tumor type	Type of study	Reference
Adenovirus ONYX-015	E1B-55 kDa gene-deletion/selective replication and lysis of p53-deficient cancer cells	Intratumoral and peritumoral injection	Recurrent advanced HNC	Phase II	Nemunaitis et al <sup>18</sup>
H101	E1B-55 kDa, E3 gene-deletion	Mouthwash Intratumoral	Premalignant oral dysplasia Various malignancies	Prospective cohort Phase I	Rudin et al <sup>19</sup> Yuan et al <sup>21</sup>
KH-901	Selective replication and lysis of telomerase-positive tumor cells using mTelo promoter (modified) and expression of GM-CSF	Intratumoral + chemotherapy Intratumoral (+ cisplatin and 5-FU) Intratumoral injection	Various malignancies HNC and esophageal cancer Recurrent HNC	Phase II Phase III Phase I	Xu et al <sup>22</sup> Xia et al <sup>23</sup> Chang et al <sup>20</sup>
HSV HF10	Lack of UL56 protein	Intratumoral Intratumoral	Recurrent HNC HNC, SCC of skin, breast cancer, melanoma	Case reports Phase I	Fujimoto et al <sup>27</sup> Ferris et al <sup>28</sup>
Onco VEX <sup>GM-CSF</sup>	ICP34.5 and ICP47 gene deletion and GM-CSF expression	Intratumoral	Breast, gastrointestinal cancers, HNC, and malignant melanoma HNSCC Stage III/IV	Phase I	Hu et al <sup>29</sup>
Reovirus Reolysin	Unmodified naturally occurring type 3 dearing reovirus – activation of Ras	Intratumoral + chemoradiation Intratumoral	Soft tissue sarcoma, HNC, melanoma, breast cancer	Phase I/II Phase I	Harrington et al <sup>32</sup> Morris et al <sup>42</sup>
RT3D	Unmodified naturally occurring type 3 dearing reovirus – activation of Ras	Intratumoral (+ radiotherapy)	Malignant gliomas Melanoma, ovarian, colorectal, lung, pancreas, esophagus, HNSCC	Phase I Two-stage Phase I	Forsyth et al <sup>43</sup> Harrington et al <sup>44</sup>
Vaccinia GL-ONCI	Insertion of the RUC-GFP, lacZ, and gusA reporter genes into the F14.5L, J2R, and A56R loci, respectively	Intravenous (+ docetaxel) Intravenous (+ gemcitabine) Intravenous (+ paclitaxel and carboplatin) Intravenous (+ paclitaxel + carboplatin)	Advanced malignancies Advanced malignancies Advanced malignancies Platinum refractory relapsed HNSCC	Phase I Phase I Phase I/II Phase III	Comins et al <sup>45</sup> Lolkema et al <sup>46</sup> Karapanagiotou et al <sup>48</sup> Multicenter (ongoing)
JX-594	Insertion of human GM-CSF, GM-CSF (CSF2), and LacZ genes into the thymidine kinase (TK) gene region causing disruption of the TK gene	Intravenous (+ concurrent cisplatin and radiotherapy) Intravenous Intratumoral	Advanced malignancies including HNC Locoregionally advanced HNC	Phase I Phase I	Jamie et al <sup>54</sup> NCT01584284 (ongoing)
Measles MV-NIS	Encodes the human thyroidal sodium iodide symporter (MV-NIS)	Intratumoral	Advanced/metastatic solid tumors Refractory primary or metastatic liver cancer	Phase I Phase I	NCT00625456 (ongoing) Park et al 2008
VSV Recombinant VSV	Gene insertion for interferon $\beta$ production	Intratumoral	Recurrent or metastatic HNSCC Refractory/intolerant hepatocellular carcinoma	Phase I Phase I	NCT01846091 (ongoing) NCT01628640 (ongoing)

**Abbreviations:** HSV, herpes simplex virus; VSV, vesicular stomatitis virus; GM-CSF, granulocyte-macrophage colony-stimulating factor; UL, long unique region; RUC-GFP, Renilla luciferase-Aequorea green fluorescent protein; 5-FU, 5-fluorouracil; HNC, head and neck cancer; SCC, squamous cell carcinoma; HNSCC, head and neck squamous cell carcinoma.

Table 2 Summary of oncolytic viruses in preclinical studies

Virus	Mechanism of selectivity/action	Method of administration	Tumor type	Model	Reference
Adenovirus Ad5/3-Δ24FCU1	Expression of the fusion suicide gene FCU1 that encodes a bifunctional fusion protein efficiently catalyzing the direct conversion of 5-FC	Intratumoral	HNSCC (larynx)	Xenograft mice	Dias et al <sup>10</sup>
Ad5/35	Substituting Ad5 fiber or penton with Ad35 fiber, thereby recognizing CD 46	Intratumoral	HNC, melanoma	Xenograft mice	Reddy PS et al <sup>11</sup>
HAdV5-E1B mutants	Defective expression of either E1B-19 K alone or both E1B-19 K and E1B-55 K proteins	Intratumoral	HNSCC (oral)	Syrian Hamster	Vijayalingam et al <sup>13</sup>
H101 and Ad-Endo	E1B55k-deleted Ad H101 selectively lyses cancer cells; replication-deficient adenovirus (Ad) has antiangiogenic and antitumoral effects	Intratumoral	Nasopharyngeal cancer	Xenograft mice	Liu et al <sup>14</sup>
Ad-DE1B19-RLX OBP-301	E1B19 deletion and RLX insertion in the site of E3 E1A and E1B genes are driven by the human telomerase reverse transcriptase (hTERT) promoter	Intratumoral Intratumoral (+ radiation)	HNSCC (oral) Radioresistant HNSCC	Xenograft mice Orthotopic mice	Lee et al <sup>15</sup> Takahashi et al <sup>16</sup>
HSV HSV 1716	Lack of RLI gene encoding the virulence protein ICP34.5	Intratumoral (+ chemotherapy)	Non-small-cell lung cancer	Xenograft mice	Toyoizumi et al <sup>17</sup>
HSV-IR849 + HSV-1 HF 34.5ENVE	γ134.5 gene deficiency Viral ICP34.5, Expressed by Nestin promoter and Vstat120 Expressing	Intratumoral (+ cisplatin) Intratumoral Intratumoral (+ bortezomib)	HNSCC HNSCC (oral) Ovarian cancer, HNC, glioma, and malignant peripheral nerve sheath tumor	Xenograft mice Xenograft mice Xenograft mice	Mace et al <sup>30</sup> Ogawa et al <sup>33</sup> Yoo et al <sup>34</sup>
G47Δ Reovirus RT3D	Deletions in γ34.5, ICP6, and alpha 47 Unmodified naturally occurring type 3 dearing reovirus – activation of Ras	Intratumoral Intratumoral (+ chemotherapy)	Nasopharyngeal cancer HNSCC	Xenograft mice Xenograft mice	Wang et al <sup>35</sup> Roulstone et al <sup>37</sup>
Vaccinia GLV-1h68	Insertion of LacZ, gusA, and Ruc-GFP into the TK, HA, and F14.5L loci, respectively	Intratumoral	HNSCC (salivary gland)	Orthotopic mice	Chemichenko et al <sup>60</sup>
Measles MV-NIS	Encodes the human thyroidal sodium iodide symporter (MV-NIS) Insertion of the hybrid gene of <i>Escherichia coli</i> CD/UPRT into the viral genome	Intratumoral Intratumoral (+ radiation)	HNSCC HNSCC	Xenograft mice Xenograft mice	Yu et al <sup>51</sup> Mansfield et al <sup>52</sup>
VSV VSVΔ51	Single amino acid deletion in the VSV M protein	Intratumoral Intratumoral (+ 5-FU)	HNSCC Recurrent and/or refractory HNSCC	Xenograft mice Xenograft mice	Li et al <sup>66</sup> Zaouiet et al <sup>58</sup>
NDV NDV(F3aa)-GFP	Modified F cleavage site-fusogenic virus with ability to form syncytia	Intravenous (+ radiation/ ZD6126) Intratumoral	HNC (hypopharyngeal FaDu tumor) HNSCC	Xenograft mice Xenograft mice	Alajez et al <sup>60</sup> Li et al <sup>61</sup>

**Abbreviations:** HSV, herpes simplex virus; VSV, vesicular stomatitis virus; NDV, Newcastle disease virus; 5-FC, 5-fluorocytosine; 5-FU, 5-fluorouracil; CD/UPRT, cytosine deaminase/uracil phosphoribosyltransferase; HNSCC, head and neck squamous cell carcinoma; HNC, head and neck cancer; EGFR, epidermal growth factor receptor.

paclitaxel or cisplatin has been found to exert significant antitumor effects on head and neck squamous cell carcinoma (HNSCC) cells both in vitro and in vivo.<sup>18</sup>

Though many adenoviruses have been studied in animal models, only a few of them have entered clinical trials in humans, namely ONYX 015, H101, and KH 901. ONYX-015 is an E1B-55 kDa gene deleted adenovirus engineered to replicate in and lyse p53-deficient cancer cells. However, it should be noted that ONYX-15 was later shown to lyse tumor cells independent of p53 status, and therefore, the exact mechanism in which ONYX-15 exerts its selectivity remains to be elucidated. A Phase II clinical study including peritumoral and intratumoral ONYX-015 injection in 24 patients with recurrent HNC showed highly selective tissue destruction with significant tumor regression (>50%) in 21% of evaluable patients, with no toxicity to injected normal peritumoral tissues. Interestingly, p53 mutant tumors underwent significantly more necrosis compared to p53 wild-type tumors. High-neutralizing antibody titers did not prevent infection or replication within tumors.<sup>19</sup> ONYX-015 was also found to be effective as a mouthwash in premalignant oral dysplasia, with specific cytotoxicity to cells carrying defects in p53 signaling pathways. Histologic resolution of dysplasia was observed in 7 (37%) of 19 patients, and the grade of dysplasia improved in one additional patient. Though the responses were transient, it was concluded to be a tolerable and feasible treatment modality with a favorable toxicity profile.<sup>20</sup>

KH901 is a conditionally replicating oncolytic adenovirus, which selectively replicates in and lyses telomerase-positive tumor cells and expresses granulocyte-macrophage colony-stimulating factor (GM-CSF). A Phase I trial involving 23 patients demonstrated that the intratumoral administration of KH901 in recurrent HNC patients was feasible, well tolerated, and associated with biological activity.<sup>21</sup>

H101, an E1B-deleted adenovirus, which is very similar to ONYX-015 but lacks all E3 proteins, was found to be safe when injected intratumorally in 15 patients in a dose escalation manner over 5 consecutive days. There was no observed dose-limiting toxicity or serious adverse events.<sup>22</sup> Additionally, various Phase II and Phase III clinical trials, involving a combination of H101 and standard chemotherapy, demonstrated improved overall response rates in HNC.<sup>23,24</sup> These studies eventually led to China's State Food and Drug Administration approving Shanghai Sunway Biotech's genetically modified adenovirus-H101 in November 2005, the first of its kind in oncolytic virotherapy for the treatment of HNC.<sup>25</sup>

## Oncolytic herpes simplex viruses

Herpes simplex virus (HSV) is a double-stranded DNA virus surrounded by a capsid and enveloped in a lipid bilayer. The HSV consists of both a long unique region (UL) containing 56 genes and a short unique region (US) containing 12 genes. Entry is mediated through receptor-mediated endocytosis involving fusion of the envelope membrane and host membrane, allowing viral entry through pores.<sup>26</sup> Once entry is achieved, HSV hijacks the host machinery for viral replication. The host antiviral defense machinery would normally inhibit production of virions through phosphorylation of protein kinase R (PKR) and downstream phosphorylation of the translation initiation factor, eIF2 $\alpha$ , effectively shutting off protein translation. However, viral protein, ICP 34.5, antagonizes PKR by directly dephosphorylating eIF2 $\alpha$ . ICP 34.5 is also important for downregulating the immune response by downmodulation of MHC (major histocompatibility complex) II expression. Deletion of ICP 34.5 results in the inability of HSV to replicate in normal cells. However, in cancer cells that have upregulated Ras, which inhibits the phosphorylation of PKR,<sup>27</sup> this pathway is already bypassed, allowing translation of viral proteins and production of virions, leading to lysis of the cancer cell. HSV is also able to evade the immune system through expression of ICP 47 which inhibits MHC class I from binding antigen to TAP (transporter associated with antigen processing). This prevents a cytotoxic T-cell response and clearance of the virus. Oncolytic herpes simplex viruses (oHSVs) are found to be highly oncolytic to tumor cells and are either naturally occurring mutants or genetically engineered by deleting virulence-dependent proteins, such as those mentioned earlier, allowing for selective replication in tumor cells while providing safety to normal bystander cells. The first oHSV, which was engineered with a deletion in thymidine kinase, was shown to be active in cancer in 1991 in a high-grade glioma mouse model.<sup>28</sup> Since then, oHSV has been investigated in many solid tumors including HNC.

HF10 is a spontaneously occurring mutant form of the HSV-1 virus lacking the UL56 protein, which is thought to be important for cytoplasmic transport and release of virions. An early proof of principle study showed that intratumoral injections in two patients with metastatic subcutaneous nodules were safe and demonstrated efficient spread and cell death in the tumor.<sup>29</sup> At the 2014 American Society of Clinical Oncology (ASCO) meeting, Ferris et al<sup>30</sup> demonstrated that multiple intratumoral injections of HF-10 in refractory and superficial cancers (HNC, squamous cell carcinoma of skin, breast cancer, and melanoma) were safe in an ongoing

Phase I study, with most common adverse events of flu-like symptoms and pain at injection site. Follow-up of this study will be interesting to determine if these patients experience clinical responses.

oHSVs have been generated to express transgenes including Onco-VEX<sup>GM-CSF</sup> (JS1/34.5-/47-/GM-CSF), which in addition to deletion of ICP 34.5 and ICP 47 is an oHSV expressing GM-CSF. A Phase I study of Onco-VEX<sup>GM-CSF</sup> showed safety in solid tumors as well as local tumor activity and suggested activity in noninjected tumors as well.<sup>31</sup> Local reaction at the injection site of the latter study was dose limiting.

However, it is unlikely that intratumoral injection of oHSV alone will be sufficient for total eradication of tumor. Preclinical studies have shown possible benefit of combining oHSV with chemotherapy. Mace et al<sup>32</sup> showed that the addition of cisplatin in vitro to HSV1716 was not cytotoxic to the virus and that efficacy was in fact additive with the combination. In non-small-cell cancer cell lines, the addition of chemotherapy to HSV1716 was synergistic.<sup>33</sup> Due to preclinical efficacy, clinical studies with oHSV in combination with chemotherapy and radiation were initiated. Impressive responses were seen when Onco-VEX<sup>GM-CSF</sup> was added to standard cisplatin and radiation in locally advanced HNC.<sup>34</sup> Patients with stage III/IVA or B were enrolled and received escalating doses of virus (4 injections total) in 3 cohorts, followed by an expansion phase with a total of 17 patients treated. All patients received concurrent cisplatin 100 mg/m<sup>2</sup> every 3 weeks and 70 Gy of radiation over 35 fractions and they also underwent neck dissection at the conclusion of treatment to assess response. The pathologic complete response rate was very high at 93% in injected tumors. Just less than half of the patients were found to be positive for HPV. At median follow-up of 29 months, 100% of patients remained free of any locoregional disease. Disease-specific survival was 82.4%, relapse-free rate was 76.5%, and overall survival was 70.5%. However, it is important to note that these high rates of survival may be skewed due to neck dissection required in all patients, which is not the usual treatment in patients who had received a radiologic complete response and had not relapsed. Despite clinical efficacy seen in Phase I/II, the Phase III study, in which Onco-VEX<sup>GM-CSF</sup> was being tested in combination with definitive cisplatin and radiation vs chemotherapy and radiation alone (clinicaltrials.gov NCT01161498), was terminated early likely due to new prognostic implications of HPV status and changing landscape of HNC treatments. Amgen (Thousand Oaks, CA, USA), who now own the virus under the new name T-VEC,

are working on new strategies for the use of the virus as treatment for HNC.

Other strategies to enhance oHSV oncolysis antitumor efficacy and safety have also been studied. Dual infection with HSV-1 mutants,  $\gamma$ 134.5 gene-deficient HSV-1 R849, and HSV-1 HF (which induces cell fusion) in a human oral SCC cell xenograft model led to enhancement of the oncolytic ability of the  $\gamma$ 134.5 gene-deficient HSV-1 R849, suggesting that fusogenic viruses may be beneficial in HNC.<sup>35</sup> Combining bortezomib treatment with oHSV (34.5ENVE) in vivo significantly increased viral replication, at least partially, through Hsp90 as well as enhanced antitumor efficacy.<sup>36</sup> G47 $\Delta$ , a third-generation oHSV with deletions in  $\gamma$ 34.5, ICP6, and  $\alpha$ 47, proved to be efficacious in a nasopharyngeal cancer model in vivo.<sup>37</sup> Deletion in the latter virus makes the virus more sensitive to acyclovir and ganciclovir as an extra safety measure.

Another strategy that is being explored is copper chelation. Physiologic levels of copper are known to inhibit HSV and have been found to abolish replication and effectiveness of oHSV in preclinical models in HNC, glioma, ovarian cancer, and various other models. Plans are being made to move this strategy into Phase I trials.<sup>38</sup>

## Reovirus

Reovirus (respiratory enteric orphan virus) is a member of the Reoviridae family. Although it is not associated with a named human disease, it can infrequently cause flu-like upper respiratory tract symptoms or a mild diarrheal illness. Exposure is very common with up to 100% of healthy human adults showing seropositivity.<sup>39</sup> There are three serotypes of reovirus, which are designated type 1 (Lang), type 2 (Jones), and type 3 (Dearing). The type 3 serotype has been extensively tested as a proprietary formulation developed by Oncolytics Biotech (Calgary, Canada). Reovirus virions consist of a nonenveloped, icosahedral capsid with a double shell of proteins. It binds to JAM-1 or sialic acid receptors in the infected cell, and subsequent endocytosis initiates cleavage of the outer shell proteins to release the core into the cytoplasm. Viral RNA is then copied within the core, and mRNA is released and translated into viral proteins using the host cell machinery. Subsequently, viral particles assemble and are released for reinfection by cell lysis.

Reovirus first sparked interest as a cancer-killing therapeutic agent in the 1970s, when WT reovirus was shown to replicate in transformed, but not normal, cells.<sup>40</sup> Reovirus-resistant NIH3T3 cells became sensitive upon transformation with activated Sos or Ras, suggesting that reovirus may

exploit elements of the Ras pathway to enhance infection.<sup>41</sup> Cells overexpressing the EGFR, upstream of Ras, were also found to be sensitive to reovirus. The Ras pathway is frequently activated in cancers, either through mutation of the Ras gene or overexpression/mutational activation of the upstream EGFR or other receptor tyrosine kinases.<sup>42</sup> In HNC, up to 90% of tumors overexpress EGFR.<sup>43</sup> The mechanism by which reovirus is selective for Ras-mutated/activated cancer cells has been shown to involve PKR similar to oHSV.

The first Phase I clinical trial involving reovirus therapy studied the safety of an intratumoral injection of reovirus to accessible subcutaneous tumors in patients who had exhausted all other standard cancer treatments. Doses up to  $1 \times 10^{10}$  tissue culture infectious dose (TCID<sub>50</sub>) were administered without encountering dose-limiting toxicity and, as a secondary endpoint of the study, 61% of patients responded to the treatment (REO001). These data demonstrated that single-agent intratumoral treatment with reovirus is well tolerated with predictable side effects of headache and flu-like symptoms.<sup>44,45</sup> Subsequently, a Phase I trial of intratumoral reovirus combined with radiotherapy was conducted. A total of 23 patients received treatment, and three had HNC. The combination treatment was well tolerated, and 14 patients had either partial response or disease stabilization as their best treatment response.<sup>46</sup> Phase I studies of intravenous reovirus in patients with advanced solid tumors reported similar side effects, an increase of antireoviral antibodies with treatment, and few positive viral shedding results. A Phase I study of intravenous reovirus and docetaxel in 25 patients with various advanced cancers showed that treatment with the combination was well tolerated and a disease-control rate (combined complete response, partial response, and stable disease) of 88% was reported.<sup>47</sup> A Phase I study of reovirus in combination with gemcitabine showed that of ten patients who were evaluable for response, one had a partial response, one a minor response, and six patients had stable disease.<sup>48</sup>

Triple therapy with reovirus, cisplatin, and paclitaxel demonstrated synergistic cytotoxicity *in vitro* in both malignant and nonmalignant cell lines. In HNC cell lines, this was associated with enhanced caspase-3 and -7 cleavage, but no increase in viral replication. Triple therapy was significantly more effective than reovirus or cisplatin–paclitaxel in athymic nude mice. These data suggest that the combination of reovirus plus platinum–taxane doublet chemotherapy has significant activity in HNC.<sup>49</sup> In a recently completed Phase I/II study, a platinum–taxane doublet regimen in combination with reovirus was tested. Treatment was well tolerated, and a dose was recommended for subsequent Phase II evaluation

(full dose chemotherapy plus  $3 \times 10^{10}$  TCID<sub>50</sub> on days 1–5). In the Phase I part of the study, treatment responses were particularly impressive in patients with incurable or relapsed/metastatic HNC. Of 19 heavily pretreated patients, there was 1 complete response, 6 partial responses, and 2 clinically meaningful responses (resolution of cutaneous disease). The median overall survival was an impressive 8.9 months.<sup>50</sup> These data have led to a randomized, multicenter Phase III study of paclitaxel and carboplatin with or without intravenous reovirus in patients with platinum-refractory relapsed HNSCC.

## Poxviruses with a focus on vaccinia virus

The poxviruses are a group of viruses that can infect both vertebrates and invertebrates. Several poxviruses have been tested in HNC cell lines including myxoma, vaccinia, raccoonpox, and tanapox viruses. Preclinically, using cell-based assays, vaccinia virus was shown to have the greatest oncolytic activity.<sup>51</sup> Utilizing the vaccinia virus as an oncolytic viral therapy is attractive due to its extensive safety profile, its use for eradication of small pox, its ability to infect a wide variety of hosts, and its potent oncolytic ability selective to cancer cells. Vaccinia virus has been modified for safety including GLV-1h68, which is an attenuated, replication-competent vaccinia virus. This virus has been shown to be oncolytic to salivary gland and HNC cell lines and resulted in a rapid tumor regression in mouse xenograft and orthotopic models.<sup>52,53</sup> In a human xenograft model in nude mice, radiation in combination with vaccinia virus resulted in higher rates of tumor regression,<sup>54</sup> suggesting that further exploration of this combination may be efficacious clinically. Vaccinia viruses have also been engineered to contain transgenes, including the vaccinia virus containing the endostatin–angiostatin fusion gene (VVhEA), which was shown to be superior to ONYX-015 and the control vaccinia virus *in vivo*.<sup>55</sup>

Clinically, JX-594 or Pexa-Vec (Sillajen, Busan, South Korea), which is a thymidine-kinase-deleted vaccinia virus plus GM-CSF, has been tested in solid tumors including HNC in a Phase I study (clinicaltrials.gov, NCT00625456). Results from this study have yet to be published, but at least in hepatocellular carcinoma, the decision has been made to move forward to Phase II based on responses in this subset. Interestingly, the vaccinia virus, GL-ONC1, which is a genetically modified virus for improved safety (deletion of thymidine kinase) and that contains an imaging transgene (genelux, San Diego, CA, USA) is currently being tested intravenously in combination with cisplatin and radiation in

HNC based on preclinical evidence to evaluate the safety of this combination (clinicaltrials.gov, NCT01584284). Prior Phase I data as a single agent were presented at ASCO 2012 and showed safety data and specific tumor delivery in multiple tumor types including HNC.<sup>56</sup>

## Paramyxoviridae

The measles virus is a single-stranded, negative-sense, enveloped RNA virus in which humans are the natural host. MV-NIS, which is a genetically modified form of the oncolytic MV-Edm virus and contains the sodium iodide symporter (NIS), has been tested preclinically both in anaplastic thyroid cancer cells and in HNC cell lines.<sup>57–59</sup> MV-NIS was shown to have oncolytic efficacy in both anaplastic thyroid cancer cell lines and xenografts, and efficacy could be boosted with cyclophosphamide, radioiodine (<sup>131</sup>I), as well as a Chk-1 inhibitor (SAR-020106) in HNC cell lines *in vitro*. These preclinical data have led to a Phase I clinical study, investigating intratumoral MV-NIS in HNC (clinicaltrials.gov NCT01846091).

Another genetically modified measles virus that was targeted to EGFR, which as mentioned earlier is highly expressed in HNC, and that contains a bifunctional enzyme cytosine deaminase/uracil phosphoribosyltransferase (CD/UPRT) has also been tested in HNC *in vitro* and *in vivo*.<sup>60</sup> CD/UPRT converts 5-fluorocytosine into the chemotherapeutic 5-FU, which is often used in HNC. Interestingly, intratumoral injection of this virus resulted in cytotoxic cell death of the tumor, but also a bystander effect on noninfected tumor cells likely through 5-FU toxicity. Per authors, a Phase I clinical trial is planned.

The Newcastle disease virus (NDV) is a paramyxovirus and has been genetically modified to enhance tumor selective killing. The naturally occurring NDV is a single-stranded RNA virus, which was shown to lyse tumor cells as early as 1964 and is advantageous in that the natural virus is pathogenic in birds but only causes mild flu-like symptoms in humans. NDV(F3aa)-GFP is a genetically modified NDV that is fusogenic allowing syncytia formation and has been tested in the treatment of HNC both *in vitro* and *in vivo*.<sup>61</sup> NDV(F3aa)-GFP was found to be highly tumor cytotoxic in the intratumoral model without associated toxicity.

## Vesicular stomatitis virus

Vesicular stomatitis virus (VSV) has been shown to have some oncolytic activity. Unfortunately, many tumors are resistant to VSV, prohibiting its use. Interestingly, one study in HNC cell lines showed that Janus kinase (JAK) 1/2 inhibitors reversed the resistance, resulting in enhanced

viral activity.<sup>62</sup> This may be one strategy in boosting VSV efficacy and transition to clinical studies. Another mutant virus, VSVΔ51 (single mutation in M protein), was tested in the human FaDu hypopharyngeal SCC model either as a single agent, combined with radiation therapy, or with the vascular disrupting agent ZD6126 which all demonstrated enhanced efficacy of the virus.<sup>63</sup> There currently is one Phase I clinical trial in liver cancer in which the VSV is genetically engineered to express interferon  $\beta$ , which alone has efficacy in hepatocellular carcinoma (NCT01628640).

Other oncolytic viruses continue to emerge in the treatment of HNC, and further modifications of these viruses hold promise for enhanced efficacy. Although there have been failures and some of the Phase I studies have been withdrawn, there continues to be promising new developments.

## Oncolytic virotherapy drawbacks and hurdles

Several obstacles continue to make generating effective oncolytic viruses difficult. Although deletion of certain genes are necessary for tumor selectivity, some of these mutations result in decreased antitumor efficacy either due to inefficient entry, replication, or lysis of tumor cells, some examples of which were already described. It remains difficult to both genetically modify the viruses for safety while continuing to enhance efficacy. Other hurdles include maintaining efficient viral spread and avoidance of the host immune system.

## Virus delivery and spread

The tumor microenvironment is critical in promoting malignant cell growth and progression, as well as restricting virus spread especially in areas of fibrosis and necrosis. In addition, viruses are larger than other anticancer agents such as chemicals and antibodies. The matrix metalloproteinases (MMPs) are proteolytic enzymes that degrade the extracellular matrix and are essential for tumor spread and neovascularization. Oncolytic viruses with genes that encode MMP inhibitors have shown good results in delaying tumor growth and angiogenesis,<sup>64</sup> providing one mechanism to overcome the microenvironment hurdle. In addition, coadministration of the enzyme hyaluronidase with oncolytic Ads during intratumoral injection was studied by Ganesh et al.<sup>65</sup> Hyaluronidase degraded the major constituent of the extracellular matrix, hyaluronan, resulting in enhanced virus spread *in vivo*. Also, induction of cancer cell death with an apoptosis-inducing agent prior to injection of oncolytic HSV could result in effective virus spread.<sup>66</sup> Okunaga et al<sup>67</sup> showed that ultrasound with microbubbles promoted the entry



of HSV-1 into oral SCC cells and increased the number and size of the RH2 antigen-positive foci within treated tumors. This suppressed the growth of nude mouse tumors. This method may be useful for enhancing the antitumor ability of oncolytic HSV-1 for HNC.<sup>67</sup>

Elevated interstitial hydrostatic pressure as a result of fibrosis and vessel abnormalities poses another physical barrier to successful virus delivery.<sup>68</sup> The viruses could escape by drainage into the circulation or back through the injection site, resulting in reduced efficacy and increased systemic toxicities. Price et al<sup>69</sup> recently demonstrated that a silk-elastin-like protein polymer (SELP) gel matrix significantly potentiates the efficacy of intraoperative oncolytic vaccinia therapy following incomplete tumor resection. The SELP gel matrix presents many advantages, including prolonged physical contact of virus against the tumor surface, effective viral infection of the tumor surface, protection of viral integrity within the SELP gel, and slow release of virus as the SELP gradually biodegrades over time.<sup>69</sup>

A virus that has successfully entered the cell needs to replicate for efficient cell lysis and virus spread. The protein p21CIP1/WAF normally inhibits cyclin-dependent kinase 2 and blocks the progression of the cell cycle from G<sub>1</sub> to S phase. siRNA knockdown of p21CIP1/WAF was shown to increase Ad replication and oncolysis by Shiina et al.<sup>70</sup> Jung et al<sup>71</sup> evaluated antitumor efficacy by sustained delivery of oncolytic Ad containing shRNA, using SELP hydrogels for treatment of HNC. The combination of oncolytic Ad with shRNA targeted to C-Met expression resulted in antitumor efficacy. SELP hydrogels maximize the potential of Ad-mediated cancer gene therapy by spatiotemporal control and preservation of biological activity in the tumor tissue. The matrix-mediated delivery of oncolytic Ad containing shRNA has potential for therapy of HNC with maximized efficacy and reduced toxicity.<sup>71</sup>

Yoo et al<sup>38</sup> demonstrated that antiangiogenic strategies increased viral replication and promoted systemic administration of oHSV. ATN-224 is a second-generation analog of tetrathiomolybdate which is used to treat Wilson's disease. ATN-224 and tetrathiomolybdate are copper chelators that also inhibit superoxide dismutase leading to potent antiangiogenic and antitumoral effects in addition to increasing oHSV efficacy.

## Host immune response against the virus

Intravenous virus delivery could be affected by neutralizing antibodies, complement activation, poor virus escape from the vascular compartment, as well as nonspecific uptake by

other tissues such as the liver and spleen. For Ad, adhesion to blood cells could also lead to therapeutic hindrance.<sup>72</sup> To modify the immune response in favor of virus replication and tumor lysis, one method is to use an immunosuppressive agent, such as cyclophosphamide, that has been shown to improve virus spread and antitumoral efficacy.<sup>73</sup> Antibodies prevent possible toxicity,<sup>74</sup> but they also reduce oncolytic efficacy. Preexisting antibodies decrease virus spread after intravenous delivery,<sup>75</sup> but have a lesser effect on intratumoral injection.<sup>76</sup> To circumvent these antibodies, plasmapheresis can be used to deplete the antibodies prior to virus injection. Another way is to use viral strains with a lower prevalence of antibodies in the human population. One example is Ad11, with a reported antibody prevalence of 10%–31% compared to 45%–90% for Ad5.<sup>77</sup>

Interestingly, despite the drawback of the activation of the immune response resulting in clearance of the virus, in some situations it is advantageous, in that an immune response directed to the virus may also result in tumor cell killing. This has led to genetic modifications of the oncolytic viruses as described earlier to produce immune growth factors. It continues to be a challenge to maintain a balance of viral potency and propagation while taking full advantage of the host immune response directed to tumor.

## Conclusion

Oncolytic viruses are promising as future HNC therapy, both alone and in combination with standard treatments. With the discovery of immunotherapy, and the activation of the immune response by viral oncotherapy, it will be interesting to determine the impact of these therapeutic combinations. The results of many ongoing clinical studies are anxiously anticipated to understand the potential use of these treatments in the future.

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