World Journal of Otorhinolaryngology-Head and Neck Surgery (2016) 2, 84—89

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/WJOHNS; www.wjent.org

REVIEW ARTICLE

The current status of oncolytic viral therapy for head and neck cancer

Matthew O. Old [a,*], Trisha Wise-Draper [b], Chadwick L. Wright [c], Balveen Kaur [d], Theodoros Teknos [a]

[a] *Department of Otolaryngology — Head and Neck Surgery, The James Cancer Hospital and Solove Research Institute, Wexner Medical Center at The Ohio State University, USA*
[b] *Division of Hematology-Oncology, Department of Internal Medicine, University of Cincinnati, USA*
[c] *Wright Center of Innovation in Biomedical Imaging, Division of Nuclear Medicine and Molecular Imaging, Department of Radiology, The Ohio State University Wexner Medical Center, USA*
[d] *Department of Neurological Surgery, Dardinger Laboratory for Neuro-Oncology and Neurosciences, The Ohio State University Comprehensive Cancer Center, USA*

Received 5 April 2016; accepted 11 May 2016
Available online 22 July 2016

KEYWORDS
Head and neck squamous cell carcinoma;
Oncolytic viruses;
Clinical trials;
Novel therapeutics

Abstract *Objective:* Cancer affects the head and neck region frequently and leads to significant morbidity and mortality. Oncolytic viral therapy has the potential to make a big impact in cancers that affect the head and neck. We intend to review the current state of oncolytic viruses in the treatment of cancers that affect the head and neck region.
Method: Data sources are from National clinical trials database, literature, and current research.
Results: There are many past and active trials for oncolytic viruses that show promise for treating cancers of the head and neck. The first oncolytic virus was approved by the FDA October 2015 (T-VEC, Amgen) for the treatment of melanoma. Active translational research continues for this and many other oncolytic viruses.
Conclusion: The evolving field of oncolytic viruses is impacting the treatment of head and neck cancer and further trials and agents are moving forward in the coming years.

* Corresponding author. The James Cancer Hospital and Solove Research Institute, Wexner Medical Center at The Ohio State University, Department of Otolaryngology-Head and Neck Surgery, Starling Loving Hall, B217, 320 West 10th Ave, Columbus, OH 43212, USA.
E-mail address: matthew.old@osumc.edu (M.O. Old).
Peer review under responsibility of Chinese Medical Association.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.wjorl.2016.05.009

Introduction

Cancers affect the head and neck region frequently and involve the skin, mucosal surfaces, sinuses, salivary glands, and endocrine system. There is a wide survival rate depending on the disease entity but melanoma, advanced thyroid cancers, and head and neck squamous cell carcinoma (HNSCC) still cause significant morbidity and mortality. Melanoma accounts for a minority of the cancers at 4% but causes a majority of skin cancer deaths.[1] The SEER database estimates there were over 73,000 new melanomas diagnosed in the United States in 2015 with 9940 deaths that year from the disease.[1] Stage III and IV melanoma carry a significant morbidity and mortality with a 5-year survival rate around 40% and 15%—20% respectively.[2] Advanced thyroid cancer carries a significant morbidity and mortality as well. Stage IV papillary and follicular thyroid cancer have a 50% 5-year survival rate while stage IV medullary and anaplastic thyroid cancer carry grim statistics with survival rates of 28% and 7%, respectively.[2] HNSCC occurs in the oral cavity, oropharynx, larynx, and hypopharynx is the sixth most common cancer worldwide and the five-year survival rates are among the worst of the major cancers. There are over 35,000 new cases of oral and oropharyngeal squamous cell carcinomas (OPSCC) diagnosed in the United States each year, resulting in 12,000 deaths.[3] Currently, patients with locally advanced HNSCC are treated with surgery, radiotherapy, chemotherapy, and molecular targeted therapies.[4] Despite advances in therapy, the morbidity and mortality of HNSCC remains high with 50% of these patients relapse and develop advanced and metastatic disease. Despite significant improvements in the therapy, the long-term survival rates in patients with advanced stages have not significantly increased. Treatment often leads to severe and permanent functional deficits with a negative impact on patients' quality of lives.[4] There is a great need for the development of novel therapies to improve survival of recurrent and metastatic head and neck cancer (HNC) patients while limiting treatment-related toxicities. Oncolytic viruses are targeted agents with the potential to fill this role for the treatment of HNC patients. Oncolytic viruses selectively replicate in tumor cells naturally or via deletion of critical virulence proteins that are required for replication in normal host tissue. This review will focus on the oncolytic viruses that have shown promise for the treatment of HNC.

Oncolytic viruses for HNC

Oncolytic virotherapy has been injected intratumorally and intravenously with excellent safety profiles in multiple human trials. Head and neck cancers are favorable solid tumors for both methods, but particularly intratumoral injection due to the proximity of clinically apparent disease. Multiple viruses have been used with varying degrees of success including herpes viruses, reovirus, adenoviruses, measles, vaccinia and a variety of others. There are many clinical trials studying oncolytic viruses and very few have demonstrated dose limiting toxicities. Talimogene laherparepvec (T-VEC, Amgen) has showed promise in treating head and neck squamous cell carcinoma and melanoma and was recently approved by the FDA for the treatment of melanoma.[5] It is the first oncolytic virus approved by the FDA and more are anticipated to follow this track. H101 is oncolytic adenovirus tested in Phase I—III trials and is approved for use by China's Food and Drug Administration for the treatment of advanced head and neck cancers.[6]

Oncolytic herpes viruses

Oncolytic herpes simplex viruses (oHSV) are double-stranded enveloped DNA viruses with naturally occurring mutations or are genetically engineered to specifically replicate in tumor tissue and avoid infection and propagation in normal cells.[7] Wildtype HSV hijack's the host machinery for replication which is combatted by phosphorylation of PKR and downstream phosphorylation of the translation initiating factor, eIF2α. HSV's viral protein ICP 34.5 has 2 important functions allowing it to replicate. It antagonizes PKR by directly dephosphorylating eIF2α and down-regulates the immune response by decreasing major histocompatibility complex II (MHC II) expression. Most oHSV typically have deletion of ICP 34.5 that effectively abolishes their ability to replicate in normal cells. Cancer cells typically have upregulated Ras which inhibits phosphorylation of PKR, allowing OV replication.

oHSV have shown anti-tumor efficacy *in vitro* and *in vivo* animal models of head and neck cancers.[8—10] In clinical trials investigating safety of oHSV administered to patients, no dose-related toxicities were identified.[8] However, complete responses or therapeutic efficacy have rarely been observed so significant improvements in oHSV therapy are necessary. Currently there are three HSV-1 derived oncolytic viruses that are being tested for safety and efficacy in patients with head and neck cancers (Table 1).

HF10 is a naturally occurring mutant form of HSV-1 that lacks the UL56 protein which regulates cytoplasmic transport and release of virions. It has been tested in a phase I trial of intratumoral injections in multiple tumors including head and neck SCC, melanoma, skin SCC, and others. The most common side effects were flu-like symptoms and pain at the injection site.[11] The trial is completing enrollment so the data is pending. It is now being tested in a phase II trial in combination with ipilumumab for advanced stage melanoma (Table 1).

Another oHSV that is now FDA approved under the name of Imlygic as of October 2015 is T-VEC (talimogene laherparepvec, Amgen; formerly Onco-VEX$^{GM\text{-}CSF}$).[5] T-VEC has deletions of ICP 34.5 and ICP 47 but expresses granulocyte macrophage colony stimulating factor (GM-CSF). It has undergone extensive testing in clinical trials in head and neck SCC and melanoma. A phase I/II study added T-VEC to standard cisplatin and radiation for the treatment of advanced stage III/IV head and neck squamous cell carcinoma.[8] All patients had post-treatment neck dissections. Median follow up was 29 months with 100% patient free of locoregional disease and a disease specific survival of 82.4% and overall survival rate of 70.5%. Pathologic complete response in the neck dissections were 100%. It should be noted that these results must be viewed in caveat that all patients had a post-treatment neck dissection which is not

the standard of care, particularly in those with a complete response to the therapy.

The agent has impressive results in the treatment of advanced melanoma in which 16.3% patients had a response to intra-tumoral injection for over 6 months in comparison to 2.1% in those not receiving the drug. This led to its approval by the FDA.[5] It is now being tested in multiple trials (Table 1) for recurrent or metastatic melanoma with and without ipilumumab (NCT01740297). The phase Ib portion of the study evaluates the safety of talimogene laherparepvec in combination with ipilimumab and the phase II is a randomized study that evaluates the safety and efficacy of talimogene laherparepvec in combination with ipilimumab versus ipilumumab alone. A phase Ib/III study of TVEC in combination with pembrolizumab is in the activation phases worldwide.[12]

HSV1716 is an oHSV derived from a deletion in the RL1 gene encoding for ICP 34.5. It has been shown to be safe with direct intra-tumoral injection into patients with gliomas, HNSCC, and melanoma. A current phase I trial (NCT00931931) is being conducted in relapsed or refractory non-CNS solid tumors in children and adults. Patients who respond to one injection will have an option for a second injection. All data is pending.

Oncolytic reovirus (respiratory enteric orphan virus)

Reovirus is a non-enveloped RNA virus that is ubiquitous with 100% of adults showing sero-positivity.[13] It has long been an interest in the oncolytic field and has been tested in many phase I, II, and III trials in head and neck and other tumors. Normal cells will shut down protein synthesis via an activated PKR will trigger events that lead to activated EIF2α. Cancerous cells with mutated or activated Ras have been shown to be sensitive to reovirus replication and killing.[14,15] There are 3 different serotypes and type 3 (Dearing) has been the most developed and tested (Oncolytics Biotech; Calagary, Canada). The first phase I (REO001) evaluated the safety of intratumoral injection. There were no dose limiting toxicities and 61% patients responded to treatment with only headaches and flu-like symptoms.[16,17]

A phase I/II trial combed reovirus with a platinum-taxane doublet regimen in incurable or relapse/metastatic HNC. The responses were impressive and the median overall survival was 8.9 months.[18] This led to a randomized phase III study (REO 018; NCT01166542) of paclitaxel and carboplatin with or without reovirus in this patient's with platinum − refractory, taxane naïve relapsed or metastatic HNSCC. The study has completed and the company announced that 167 patients were enrolled. Locoregional disease patients treated on the intervention arm showed a statistically significant progression free and overall survival benefit versus the control arm ($P = 0.0072$, $HR = 0.5460$; $P = 0.0146$, $HR = 0.5099$ respectively).[19] For patients with metastatic disease ($n = 47$), the numbers were not powered enough to detect a difference in PFS or OS, but they noted a significant tumor stabilization or shrinkage ($P = 0.021$).

Oncolytic adenoviruses

Adenoviruses were one of the first engineered viruses to be tested in clinical trials. They are double stranded non-enveloped DNA viruses that are cytotoxic unmodified. Most oncolytic viruses contain modifications including the deletion of the p300/cbp-binding or the pRB-binding region of EA1 resulting in tumor selective replication and oncolysis. Others have a deletion of the E1B −55 kDa gene resulting in the loss of p53 degradation conferring specificity to tumors.[20–22] Many have been tested pre-clinically but very few have been tested in clinical trials except for H101, Onyx 015 and KH901.

Table 1 Current oncolytic clinical trials for cancers affecting the head and neck.

ClinicalTrials.gov ID	Oncolytic agent	Combination	Phase	IT versus IV	Disease
NCT02428036	TBI-1401(HF10) (HSV-1)	No	Phase I	IT	SCC skin Melanoma
NCT02272855	TBI-1401(HF10) (HSV-1)	Ipilimumab	Phase II	IT	Stage IIIB, Stage IIIC, or Stage IV Unresectable or Metastatic Malignant Melanoma
NCT00769704	TVEC (HSV-1)	No	Phase III	IT	Unresectable Stage IIIb, IIIc and IV Disease
NCT01368276	TVEC (HSV-1)	No	Maintenance	IT	Unresectable Stage IIIb, IIIc and IV Disease
NCT01740297	TVEC (HSV-1)	Ipilimumab	Phase Ib	IT	Unresectable melanoma
NCT00931931	HSV1716 (HSV-1)	No	Phase I	IT	Refractory non-central nervous system (Non-CNS) solid tumors
NCT01166542	Reolysin (Reovirus)	Paclitaxel and Carboplatin	Phase III	IV	Metastatic or recurrent SCC
NCT01846091	MV-NIS (Measles virus)	No	Phase I	IT	Recurrent or metastatic SCC
NCT01584284	GL-ONC1 (Vaccinia)	Cisplatin and radiation	Phase I	IV	Locally advanced H&N SCC
NCT00429312	JX-594 (Vaccinia)	No	Phase I/II	IT	Stage III/IV unresectable melanoma
NCT00438009	CVA21 (Coxsackievirus Type A21)	No	Phase I	IT	Stage IV melanoma

IT, intratumoral; IV, intravenous; SCC: squamous cell carcinoma.

H101 is an E1B-deleted adenovirus used primary via intratumoral injection with favorable safety profile. It was found to be safe and effective in Phase I, II and III trials involving combination of the agent with chemotherapy in head and neck cancers (Yuan, Xia, Xu).[23–25] This led to the approval of this agent by China's State Food and Drug Administration for the treatment of head and neck cancer in 2005.[8] There are currently no US trials with the agent.

Onyx 015 is a E1B-55 kDa deleted virus designed to replicate and destroy p53-deficient cancer and has been tested in Phase I and II head and neck trials. A phase II trial of Onyx 015 evaluated safety and response of OV injected peritumoral and intratumor in 24 patients. Greater than 50% showed significant tumor response, most pronounced in p53 mutant tumors.[26] It has also been tested as a mouthwash for the treatment of premalignant oral dysplasia with one-third of patients having resolution of their lesions with a favorable toxicity, feasibility, and safety profiles.[27]

KH901 selectively replicates in telomerase-positive tumor cells and expresses GM-CSF. A phase I trial of intratumoral administration of this OV in advanced head and neck cancers demonstrated safety, feasibility, and biologic activity.[28]

Oncolytic measles viruses

Measles virus is a single stranded enveloped RNA virus that has extensive preclinical efficacy with modified versions.[29] MV-NIS is one particular virus which is genetically modified and contains the sodium iodide symporter with good preclinical efficacy in head and neck and anaplastic carcinoma.[30–32] MV-NIS is being studied in a phase I trial (Table 1; NCT0186091) in recurrent or metastatic squamous cell carcinoma and results are pending.

Other oncolytic viruses

A variety of poxviruses have been studied preclinically but few have made it to trials for head and neck cancers. Vaccinia virus is the most clinically-vetted of the poxviruses as not only did it eradicate small pox but it also has a selective affinity for killing cancer cells. NCT01584284 assessed in a Phase I format the safety of an altered vaccinia virus (GL-ONC1) in combination with standard of care cisplatin and radiation for locally advanced SCC. GL-ONC1 is modified via the deletion of thymidine kinase and also includes an imageable transgene. The study was completed in 2015 and final results are pending. It is reported to be well-tolerated at therapeutic dose levels, with documented evidence of antitumor activity in multiple solid tumors including HNC.[33]

JX-594 (Developed by Jennerex and Pexa-Vec, Sillajen, Busan, Korea) is a vaccinia virus with thymidine kinase deleted but GM-CSF has been added. A phase I trial for advanced solid tumors including HNC was completed (NCT00625456) and results are pending. Another phase I/II trial is ongoing with intratumoral injection of JX-594 in stage III/IV melanoma patients (NCT00429312), Evidence from the phase I portion demonstrated positive responses.[34] Responses were seen in most patients at both the injected and non-injected lesions. One patient had a partial response and while another had a complete response. Response was noted of both injected tumors (in 5 of 7 patients) and at least one non-injected tumor. Efficacy and gene expression was detected in all patients despite previous vaccinia vaccination.

Coxsackieviruses (CV) are ubiquitous and typically cause self-limiting viral upper respiratory infections. CVA21 is a naturally occurring form of the virus that is being tested in a phase I trial (NCT00438009) for Stage IV melanoma (Table 1). It is injected intratumorally into superficial lesions.[35] CVA21 uses two receptors (intracellular adhesion molecule 1 (ICAM-1) and decay accelerating factor) for infection which are expressed on melanoma cells as well as other cancers.

Molecular imaging using viruses

Measuring response in clinical trials for oncolytic viral therapy is done by standard RECIST criteria. However, incorporating novel imaging techniques into trials is beginning to occur. It has been described for over the last 15 years that non-invasive and quantitative imaging assessment of viral-mediated thymidine kinase activity can be performed using conventional nuclear medicine technology (e.g., planar GC imaging, SPECT, SPECT/CT and PET/CT) and a substrate (i.e., 2′-fluoro-2′-deoxy-1β-D-arabinofuranosyl-5-iodouracil or FIAU) radiolabeled with Fluorine-18 (^{18}F, positron-emitter, half-life of ~110 m), Iodine-123 (^{123}I, gamma emitter, half-life of 13.2 h), Iodine-124 (^{124}I, positron-emitter, half-life of ~4.2 d), Iodine-125 (^{125}I, weak gamma emitter, half-life of 60 d) or Iodine-131 (^{131}I, gamma and therapeutic beta minus emitter, half-life of ~8 d).[36–41] Originally developed as an antiviral for herpes and hepatitis, FIAU is a thymidine nucleoside analog which is initially phosphorylated by viral thymidine kinases. FIAU-monophosphate is further phosphorylated into FIAU-diphosphate and − triphosphate by other intracellular kinases. FIAU-triphosphate becomes incorporated into DNA and eventually inhibits viral replication.[36,38,42,43] Interestingly, mammalian thymidine kinases exhibit very low levels of FIAU phosphorylation when compared with HSV-1-tk.[38]

During its initial clinical evaluation as an antiviral therapy for chronic hepatitis B, long-term daily administration of FIAU lead to lactic acidosis and multi-organ toxicity.[44] On the other hand, single nuclear medicine administration of radiolabeled FIAU guided by the tracer principle requires a significantly lower dose (lower by 10^{-5}—10^{-6}) for imaging HSV-1-tk activity.[42,45] Tracer doses of ^{124}I-FIAU have already been used successfully for PET/CT imaging of bacterial thymidine kinase activity in patients with active musculoskeletal infections with no evidence of toxicity.[46] Several pre-clinical studies have demonstrated the utility of FIAU imaging of intracellular thymidine kinase expression/activity and especially for herpes simplex virus 1 thymidine kinase (HSV-1-tk).[36] Depending on the kinetics of the process under investigation, PET imaging of HSV-1-tk can be performed using the shorter-lived ^{18}F-FIAU or the long-lived ^{124}I-FAIU.[47] FIAU does not readily penetrate the intact blood brain barrier (BBB) but can readily localize within the central nervous system under conditions of BBB disruption.[42] FIAU has been used successfully for imaging HSV-1-tk

expression in tumors mediated through various viral vectors including herpes viral, adenoviral and retroviral vectors.[42] *In vivo* and *in vitro*, accumulation of FIAU radioactivity corresponds to virally-infected/transfected viable cells.[43] HSV-1-tk activity in tumors can be detected within 30 min post injection but depending on the radioisotope used, HSV-1-tk activity can be serially imaged for hours to days following administration.[36,37]

The long-lived therapeutic ^{131}I-FIAU agent has been used pre-clinically to assess the efficacy of oncolytic viral therapy in tumors treated with ganciclovir.[38] Furthermore, bortezomib has been shown to induce thymidine kinase activity in some viral-associated tumors and this effect can be detected and monitored using diagnostic radiolabeled FIAU imaging or further exploited with therapeutic radiolabeled FIAU.[39,41] Advanced molecular imaging approaches using radiolabeled FIAU will likely prove useful for future clinical trials that employ existing and new oncolytic viral agents expressing the HSV-1-tk.[37]

Future directions

The majority of oncolytic viral research is focused on combination therapy. OV therapy alone has historically shown to be effective in preclinical studies but preliminary clinical trials have shown mixed efficacy. Thus, the field has moved towards enhancing not only the viral infection but the host's response to it. Immunosuppression with chemotherapy (cisplatin, bortezomib) or monoclonal antibodies (cetuximab) has been used in trials with safe side effect profiles and enhancement of the OV response. Anti-angiogenic strategies with copper chelation and other agents is a focus of much research to reduce the influx of the immune system, antiviral immune response, and washout effect of the virus. Histone deacetylase inhibitors combined with reovirus has shown significant synergy via up-regulation of the cell-surface viral receptor as well as some immune system responses that are being further defined. An exciting direction of OV therapy is in the field of immune stimulation with incorporation of endogenous factors (viral produced GM-CSF) or combination therapy with checkpoint agents such PD-1 inhibitors. Current trials are underway and being opened with these combinations under the theory and preclinical evidence that stimulating a robust host anti-tumor response will lead to synergy and enhancement of OV therapy.

Grant support

MOO is supported by Award Number UL1RR025755 from the National Center For Advancing Translational Sciences, The Joan Bisesi Memorial Career Development Grant, and the Department of Otolaryngology-Head and Neck Surgery, The Ohio State University. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center For Advancing Translational Sciences or the National Institutes of Health.

CWD is supported by the Clinical Scientist Training Program at the University of Cincinnati.

CLW is supported by (1) Grant no. IRG-67-003-50 from the American Cancer Society, (2) Award no. UL1TR000090 from the National Center For Advancing Translational Sciences (The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center For Advancing Translational Sciences or the National Institutes of Health), (3) Richard P. & Marie R. Bremer Medical Research Fund and William H. Davis Endowment for Basic Medical Research from The Ohio State University Medical Center (The remarks and opinions are the sole responsibility of the authors and do not necessarily reflect the views of the Davis/Bremer Research Fund or The Ohio State University Medical Center), (4) the National Institutes of Health (NIH)/National Cancer Institute (NCI), Clinical Loan Repayment Program, and (5) Wright Center of Innovation in Biomedical Imaging and Ohio TECH 10-012.

References

1. SEER database. http://seer.cancer.gov/statfacts/html/melan.html.
2. American Cancer Society. *Cancer facts & figures 2016*. Atlanta, Ga: American Cancer Society; 2016.
3. Shiboski CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 20-44 years. *Cancer*. 2005;103:1843—1849.
4. Forastiere AA, Trotti A, Pfister DG, Grandis JR. Head and neck cancer: recent advances and new standards of care. *J Clin Oncol*. 2006;24:2603—2605.
5. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm469571.htm.
6. Kelly E, Russell SJ. History of oncolytic viruses: genesis to genetic engineering. *Mol Ther*. 2007;15:651—659.
7. Le Boeuf F, Bell JC. United virus: the oncolytic tag-team against cancer!. *Cytokine Growth Factor Rev*. 2010;21:205—211.
8. Harrington KJ, Hingorani M, Tanay MA, et al. Phase I/II study of oncolytic HSV GM-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.
9. Gil Z, Rein A, Brader P, et al. Nerve-sparing therapy with oncolytic herpes virus for cancers with neural invasion. *Clin Cancer Res*. 2007;13:6479—6485.
10. Mace AT, Ganly I, Soutar DS, Brown SM. Potential for efficacy of the oncolytic Herpes simplex virus 1716 in patients with oral squamous cell carcinoma. *Head Neck*. 2008;30:1045—1051.
11. Presented at ASCO *J Clin Oncol*. 2014;32. suppl; abstr 6082.
12. http://www.prnewswire.com/news-releases/amgen-and-merck-announce-expansion-of-collaboration-to-support-studies-of-talimogene-laherparepvec-in-combination-with-keytruda-pembrolizumab-in-patients-with-head-and-neck-cancer-300090709.html.
13. Tai JH, Williams JV, Edwards KM, Wright PF, Crowe Jr JE, Dermody TS. Prevalence of reovirus-specific antibodies in young children in Nashville, Tennessee. *J Infect Dis*. 2005;191:1221—1224.
14. Vorburger SA, Pataer A, Swisher SG, Hunt KK. Genetically targeted cancer therapy: tumor destruction by PKR activation. *Am J Pharmacogenomics*. 2004;4:189—198.
15. Meurs E, Chong K, Galabru J, et al. Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. *Cell*. 1990;62:379—390.
16. Morris DG, Feng X, DiFrancesco LM, et al. REO-001: a phase I trial of percutaneous intralesional administration of reovirus type 3 dearing (Reolysin®) in patients with advanced solid tumors. *Invest New Drugs*. 2013;31:696—706.

17. Forsyth P, Roldan G, George D, et al. A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas. *Mol Ther*. 2008;16:627—632.
18. Karapanagiotou EM, Roulstone V, Twigger K, et al. Phase I/II trial of carboplatin and paclitaxel chemotherapy in combination with intravenous oncolytic reovirus in patients with advanced malignancies. *Clin Cancer Res*. 2012;18:2080—2089.
19. http://www.oncolyticsbiotech.com/news/oncolytics-biotech-inc-announces-additional-data-from-reo-018-randomized-study-of-reolysin-in-head-and-neck-cancers/.
20. Bischoff JR, Kirn DH, Williams A, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science*. 1996;274:373—376.
21. O'Shea CC, Johnson L, Bagus B, et al. Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity. *Cancer Cell*. 2004;6:611—623.
22. Fueyo J, Gomez-Manzano C, Alemany R, et al. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. *Oncogene*. 2000;19:2—12.
23. Yuan ZY, Zhang L, Li S, Qian XZ, Guan ZZ. Safety of an E1B deleted adenovirus administered intratumorally to patients with cancer. *Ai Zheng*. 2003;22:310—313.
24. Xia ZJ, Chang JH, Zhang L, et al. Phase III randomized clinical trial of intratumoral injection of E1B gene-deleted adenovirus (H101) combined with cisplatin-based chemotherapy in treating squamous cell cancer of head and neck or esophagus. *Ai Zheng*. 2004;23:1666—1670.
25. Xu RH, Yuan ZY, Guan ZZ, et al. Phase II clinical study of intratumoral H101, an E1B deleted adenovirus, in combination with chemotherapy in patients with cancer. *Ai Zheng*. 2003;22:1307—1310.
26. Nemunaitis J, Ganly I, Khuri F, et al. Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial. *Cancer Res*. 2000;60:6359—6366.
27. Rudin CM, Cohen EE, Papadimitrakopoulou VA, et al. An attenuated adenovirus, ONYX-015, as mouthwash therapy for premalignant oral dysplasia. *J Clin Oncol*. 2003;21:4546—4552.
28. 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.
29. Touchefeu Y, Khan AA, Borst G, et al. Optimising measles virus-guided radiovirotherapy with external beam radiotherapy and specific checkpoint kinase 1 inhibition. *Radiother Oncol*. 2013;108:24—31.
30. Li H, Peng KW, Russell SJ. Oncolytic measles virus encoding thyroidal sodium iodide symporter for squamous cell cancer of the head and neck radiovirotherapy. *Hum Gene Ther*. 2012;23:295—301.
31. Reddi HV, Madde P, McDonough SJ, et al. Preclinical efficacy of the oncolytic measles virus expressing the sodium iodide symporter in iodine non-avid anaplastic thyroid cancer: a novel therapeutic agent allowing noninvasive imaging and radioiodine therapy. *Cancer Gene Ther*. 2012;19:659—665.
32. Zaoui K, Bossow S, Grossardt C, et al. Chemovirotherapy for head and neck squamous cell carcinoma with EGFR-targeted and CD/UPRT-armed oncolytic measles virus. *Cancer Gene Ther*. 2012;19:181—191.
33. Presented at ASCO *J Clin Oncol*. 2012;30. suppl; abstr 2530.
34. https://clinicaltrials.gov/ct2/show/NCT00429312.
35. https://clinicaltrials.gov/ct2/show/NCT00438009.
36. Haubner R, Avril N, Hantzopoulos PA, Gansbacher B, Schwaiger M. In vivo imaging of herpes simplex virus type 1 thymidine kinase gene expression: early kinetics of radiolabelled FIAU. *Eur J Nucl Med*. 2000;27:283—291.
37. Bennett JJ, Tjuvajev J, Johnson P, et al. Positron emission tomography imaging for herpes virus infection: implications for oncolytic viral treatments of cancer. *Nat Med*. 2001;7:859—863.
38. Deng WP, Yang WK, Lai WF, et al. Non-invasive in vivo imaging with radiolabelled FIAU for monitoring cancer gene therapy using herpes simplex virus type 1 thymidine kinase and ganciclovir. *Eur J Nucl Med Mol Imaging*. 2004;31:99—109.
39. Fu DX, Tanhehco YC, Chen J, et al. Virus-associated tumor imaging by induction of viral gene expression. *Clin Cancer Res*. 2007;13:1453—1458.
40. Nimmagadda S, Mangner TJ, Douglas KA, Muzik O, Shields AF. Biodistribution, PET, and radiation dosimetry estimates of HSV-tk gene expression imaging agent 1-(2′-deoxy-2′-18F-fluoro-beta-D-arabinofuranosyl)-5-iodouracil in normal dogs. *J Nucl Med*. 2007;48:655—660.
41. Fu DX, Tanhehco Y, Chen J, et al. Bortezomib-induced enzyme-targeted radiation therapy in herpesvirus-associated tumors. *Nat Med*. 2008;14:1118—1122.
42. Jacobs A, Braunlich I, Graf R, et al. Quantitative kinetics of [124I]FIAU in cat and man. *J Nucl Med*. 2001;42:467—475.
43. Jacobs A, Tjuvajev JG, Dubrovin M, et al. Positron emission tomography-based imaging of transgene expression mediated by replication-conditional, oncolytic herpes simplex virus type 1 mutant vectors in vivo. *Cancer Res*. 2001;61:2983—2995.
44. McKenzie R, Fried MW, Sallie R, et al. Hepatic failure and lactic acidosis due to fialuridine (FIAU), an investigational nucleoside analogue for chronic hepatitis B. *N Engl J Med*. 1995;333:1099—1105.
45. Pomper MG. Letter to the editor. *Semin Nucl Med*. 2009;39:354.
46. Diaz Jr LA, Foss CA, Thornton K, et al. Imaging of musculoskeletal bacterial infections by [124I]FIAU-PET/CT. *PLoS One*. 2007;2:e1007.
47. Brust P, Haubner R, Friedrich A, et al. Comparison of [18F]FHPG and [124/125I]FIAU for imaging herpes simplex virus type 1 thymidine kinase gene expression. *Eur J Nucl Med*. 2001;28:721—729.

Edited by Jie Gao