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REVIEW

Reovirus in cancer therapy: an evidence-based review

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Derek Clements[1,*]
Erin Helson[2,*]
Shashi A Gujar[2,3]
Patrick WK Lee[1,2]

[1]Department of Pathology, [2]Department of Microbiology and Immunology, Dalhousie University, [3]Strategy and Organizational Performance, IWK Health Center, Halifax, Nova Scotia, Canada

*These authors contributed equally to this work, and are joint first authors

Abstract: Reovirus, a double-stranded ribonucleic acid virus and benign human pathogen, preferentially infects and kills cancer cells in its unmodified form, and is one of the leading oncolytic viruses currently undergoing clinical trials internationally. With 32 clinical trials completed or ongoing thus far, reovirus has demonstrated clinical therapeutic applicability against a multitude of cancers, including but not limited to breast cancer, prostate cancer, pancreatic cancer, malignant gliomas, advanced head and neck cancers, and metastatic ovarian cancers. Phase I trials have demonstrated that reovirus is safe to use via both intralesional/intratumoral and systemic routes of administration, with the most common adverse reactions being grade I/II toxicities, such as flu-like illness (fatigue, nausea, vomiting, headache, fever/chills, dizziness), diarrhea, and lymphopenia. In subsequent Phase II trials, reovirus administration was demonstrated to successfully decrease tumor size and promote tumor necrosis, thereby complementing compelling preclinical evidence of tumor destruction by the virus. Importantly, reovirus has been shown to be effective as a monotherapy, as well as in combination with other anticancer options, including radiation and chemotherapeutic agents, such as gemcitabine, docetaxel, paclitaxel, and carboplatin. Of note, the first Phase III clinical trial using reovirus in combination with paclitaxel and carboplatin for the treatment of head and neck cancers is under way. Based on the evidence from clinical trials, we comprehensively review the use of reovirus as an anticancer agent, acknowledge key obstacles, and suggest future directions to ultimately potentiate the efficacy of reovirus oncotherapy.

Keywords: virotherapy, reovirus, oncotherapy

Introduction

Oncolytic viruses (OVs) preferentially target and kill cancer cells through a process known as oncolysis, while leaving normal, untransformed cells relatively unaffected. The susceptibility of a particular cell to OV infection and subsequent oncolysis stems from the aberrant cellular signaling and defective immune responses, which are inherently common in cancer cells. Currently, numerous viruses, in their natural or modified form, have been shown to possess oncolytic properties, and thus are being studied for their potential use as anticancer agents. Some prominent examples of these OVs include reovirus,[1] Newcastle disease,[2] vesicular stomatitis,[3] vaccinia,[4] measles,[5] poliovirus,[6] herpes simplex,[7] adenovirus,[8] Maraba,[9,10] and Coxsackie.[11]

Of the viruses just mentioned, reovirus is considered a pioneer in the field of OVs, and is currently undergoing Phase I, II, and III clinical trials internationally, including in the US, the UK, Belgium, and Canada. Thus far, reovirus has been shown to kill a variety of cancers, including those of the breast, brain, lymphoma, ovarian, head and

Correspondence: Patrick WK Lee
Department of Microbiology and Immunology, Dalhousie University, 7P Charles Tupper Building, 5850 College Street, Halifax, Nova Scotia B3H 1X5, Canada
Email patrick.lee@dal.ca

neck, spine, bladder, prostate, skin, epithelium, lung, and colon.[12–24] The selective and potent oncolytic properties of reovirus have been attributed to various aberrations in cancer cells, especially those involved in activated *Ras* oncogene signaling and impaired type I IFN pathways.[1,25–30] Although *Ras* mutations occur in approximately 30% of all human cancers, aberrant signaling caused by increased Ras expression or mutations of elements upstream and downstream of Ras are prevalent in the majority of cancers, making them suitable targets for reovirus-based anticancer therapy.

Although the use of reovirus in cancer therapy stems from the oncolytic capabilities of the virus, recent studies have illustrated that reovirus additionally invokes a sequence of immunological events that ultimately overturn various tumor-induced immunosuppressive mechanisms and promotes the development of an antitumor immune response.[31–36] Studies have shown that reovirus treatment promotes the secretion of a range of proinflammatory cytokines and chemokines following administration.[31,35,37] Additionally, upon exposure to reovirus, dendritic cells (DCs) produce various proinflammatory cytokines, undergo maturation, and migrate into the tumor microenvironment along with $CD8^+$ T cells.[31,36] These reovirus-activated DCs possess the ability to prime tumor antigen-specific T cells (both in vitro and in vivo)[31] and increase the cytolytic activity of innate immune cells (natural killer [NK] cells).[36] Such a robust reovirus-mediated antitumor immunity can also protect the host against subsequent tumor challenge, even after discontinuation of therapy.[31] Collective evidence thus far suggests that reovirus-based cancer therapy targets cancer by direct oncolysis and antitumor immune activities, both of which are essential to achieve optimal tumor regression and clinical outcomes. In this context, this review focuses on preclinical and clinical studies with reovirus as a single agent or in combination therapy for treatment of cancers. We highlight key findings from the studies on reovirus oncotherapy and identify major obstacles that if appropriately managed would dictate the successful translation of reovirus oncotherapy into clinical practice.

Preclinical studies with reovirus

Hashiro et al first observed early connections with reovirus and its oncolytic properties, and found that certain tumor cells and spontaneously transformed cell lines (human and murine) have preferential susceptibility toward the cytotoxic effects of reovirus.[38] It was also noted that simian virus (SV-40)-transformed human embryonic lung cells (WI-38 cells) had increased sensitivity to reovirus cytotoxicity in comparison to untransformed WI-38 cells.[39] Although these studies revealed the oncolytic properties of reovirus, it was not until two decades later when Coffey et al published a paper in *Science* that the possibility of using reovirus as an anticancer agent was entertained.[1] This article revealed that a single intratumoral injection of reovirus could result in the regression of established v-*erbB*-transformed NIH 3T3 or human U87 glioblastoma tumors in 80% of severe combined immunodeficient (SCID) mice.[1] Furthermore, using an immunocompetent mouse model (Ras-transformed C3H-10T1/2 fibroblasts implanted into the flank of C3H mice), they also illustrated that a therapeutic regimen of multiple reovirus injections resulted in complete regression in 65% of the tumors.[1] Given the frequency of mutations in Ras and associated pathways in human cancers, as well as the relatively nonpathogenic nature of reovirus, it was evident that reovirus was an attractive anticancer agent.

Following the discovery of the oncolytic potential of reovirus, subsequent studies focused on expanding the applicability of reovirus as a single agent against multiple types of cancers in various models. A study by Hata et al found that reovirus could kill six human breast cancer cell lines (SK-BR-3, KPL4, MDA-MB-453, CRL1500, MCFT, and MDA-MB-231) but not a normal breast cell line (Hs578Bst).[12] Complementing this study, it was also illustrated that reovirus could effectively shrink MDA-MB-453 tumors in SCID mice, including tumors situated remotely from the reovirus injection site.[13]

This bilateral tumor model suggests that reovirus can be used for systemic therapy for breast cancer. In addition to breast cancer, reovirus oncotherapy is effective in brain, lymphoma, ovarian, spinal, bladder, and colon-derived cancer cells and cancer stem cells.[14,17,21,23,24,40] One particular study treated low-passage cell lines derived from nine surgically excised human gliomas, and demonstrated that ex vivo reovirus treatment killed 100% of specimens.[17] Although the majority of these preclinical studies were conducted in vitro or in SCID/nude mouse models in vivo, it was also illustrated that reovirus had beneficial anticancer effects in immunocompetent mouse models,[1,41] especially in the presence of immunosuppressant agents, such as cyclosporine A or anti-CD4/anti-CD8 antibodies.[41] Therefore, reovirus oncotherapy has the potential to be utilized for the treatment of a wide range of cancer types and may benefit more with a combination approach, especially in immunocompetent hosts.

Early studies using reovirus as a single anticancer agent showed impressive preclinical therapeutic efficacy. However, the efficacy of reovirus monotherapy was suboptimal in

immunocompetent hosts. To address this issue, reovirus was combined with various anticancer treatment options, such as chemotherapeutic agents or radiotherapy, to achieve enhanced efficacy. Interestingly, these combination studies revealed that the use of reovirus along with common chemotherapeutic agents, such as cisplatin, docetaxel, or cisplatin–paclitaxel doublet chemotherapy or radiotherapy, had synergistic effects in vitro and in vivo.[18–20,42] For example, a study conducted by Sei et al investigated the in vitro combination effects of reovirus with a variety of chemotherapeutic agents (cisplatin, gemcitabine, vinblastine, and paclitaxel) against human non-small-cell lung cancer (NSCLC) cells.[22] Additionally, strong synergistic effects were observed only in those cell lines that were sensitive to monotherapy of the respective chemotherapeutic agent.[22] In contrast, treatment of NSCLC cell lines with the reovirus and paclitaxel combination treatment was synergistic in all cell lines regardless of their sensitivity to either agent alone.[22] This enhanced treatment efficacy was suggested to be the result of accelerated apoptosis triggered by paclitaxel-mediated mitotic arrest.[22] This suggests that certain changes in programmed cell-death pathways may be advantageous in enhancing the efficacy of reovirus therapy. In agreement with this hypothesis, recent studies have also shown that activation or stabilization of programmed cell-death pathways further enhances reovirus-induced apoptosis.[15,43] In particular, actinomycin D, etoposide, or nutlin-3a have all shown enhanced reovirus-induced apoptosis via p53-dependent NF-κB activation.[15,43] Therefore, if properly chosen, a combination therapeutic approach, with lower chemotherapeutic dosages compared to monotherapy trials, could enhance reovirus-mediated cytotoxicity while reducing drug toxicity.

Clinical studies with reovirus as an anticancer agent

Since 2000, clinical-grade formulation of the type 3 Dearing strain of wild-type reovirus (Reolysin®; Oncolytics Biotech, Calgary, Canada[44]) has been tested in various clinical trials for the treatment of a multitude of cancers, such as prostate cancer,[45–47] malignant gliomas,[48–50] advanced head and neck cancers,[51–54] and metastatic ovarian cancers,[55–57]among others. There have been a total of 32 clinical trials – 15 completed and 17 ongoing – internationally in countries including Canada, the US, and the UK. Of all the clinical trials, six are in partnership with the US National Cancer Institute, and four are in partnership with the National Cancer Institute of Canada Clinical Trials Group. So far, 308 patients have been treated with reovirus in completed studies; approximately 610 patients are currently enrolled in ongoing trials, and it is expected that an additional 631 patients will be enrolled in future trials (see Table 1). Reovirus has been or will be used in 12 clinical trials as a monotherapy and in 20 clinical trials in combination with other anticancer agents.

Clinical trials with reovirus as a single agent

The first Phase I clinical trial to use reovirus was an intralesional monotherapy for the treatment of 18 patients with advanced solid tumors, including soft-tissue sarcomas, melanoma, breast cancer, and head and neck cancers.[58] This study was designed to determine the safety and tolerability of reovirus percutaneous intralesional administration in patients with prior therapeutic interventions. Patients were monitored for 6 weeks, toxicities were measured according to the National Cancer Institute Clinical Trials Group expanded common toxicity criteria, and tumor responses were measured using the Response Evaluation Criteria in Solid Tumors (RECIST). After 6 weeks, one patient showed a complete response (CR) and two demonstrated partial responses (PRs), while four had stable disease (SD) and ten showed progressive disease (PD). The results showed for the first time that reovirus administered intralesionally up to 1×10^{10} plaque-forming units as a monotherapy is safe and well tolerated without reaching dose-limiting toxicities (DLTs) or maximum tolerated dosages (the dose at which two or more subjects out of six in a dose group experience DLT). Based on the safety data from this first trial, a translational study was designed to assess the oncolytic activity (ie, the efficacy) of reovirus in preclinical models and in six prostate cancer patients.[45] In this study, the prostate cancer patients first received a single reovirus injection of 1×10^{7} plaque-forming units, and 3 weeks later underwent prostatectomies. Importantly, the immunohistochemical analysis of resected prostate tissues from these patients revealed a preference of reovirus to infect cancerous tissue rather than noncancerous tissue. The same analysis also demonstrated apoptosis and lymphocyte infiltration in reovirus-treated cancerous tissues. Together, the results from this trial supported the preclinical findings that reovirus preferentially targets and kills cancer cells and also promotes immunomodulation in the cancer milieu. Two subsequent combination Phase I/II studies were performed using local administration of reovirus for recurrent malignant gliomas, and confirmed that intratumoral administration of reovirus is well tolerated as a monotherapy.[48,49]

Next, two clinical trials investigated whether reovirus could be safely administered systemically to treat advanced

Table 1 Summary of completed, ongoing, and actively recruiting clinical trials using reovirus

Trial number[a]	Phase	Status	No of patients[b]	Cancer type(s)	Mode of administration	Dose of reovirus[c]
REO 001	I	C	18	Soft-tissue sarcoma, head and neck, melanoma, breast, other advanced solid tumors	ITu	1×10^{10} PFU once weekly
REO 002	Translational	C	6	Prostate	ITu	1×10^{7} PFU once
REO 003	I/II	C	12	Glioblastoma multiforme, anaplastic astrocytoma, anaplastic oligoastrocytoma	ITu	1×10^{9} $TCID_{50}$ once
REO 004	I	C	18	Ovarian, colon, breast, cervical, leiomyosarcoma, carcinoid, prostate, NSCLC	iv	3×10^{10} once $TCID_{50}$ every 4 weeks
REO 005	I	C	33	Head and neck, prostate, colorectal, pancreas, upper gastrointestinal, melanoma, soft-tissue sarcoma, bladder, NSCLC, renal, endometrial	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 4 weeks
REO 006	I	C	23	Melanoma, head and neck, squamous cell carcinoma of the skin, lung, ovarian, colorectal, esophagus, pancreas, unknown primary	ITu	1×10^{10} twice $TCID_{50}$ every week up to 6 injections
REO 007	I/II	C	15	Anaplastic astrocytoma, glioblastoma multiforme	ITu	1×10^{10} $TCID_{50}$ once over 72 hours
REO 008	II	C	16	Melanoma, colorectal, gastric, pancreatic, ovarian, lung, cholangio-carcinoma, sinus, thyroid	ITu	1×10^{10} $TCID_{50}$ twice
REO 009	I	C	16	NSCLC, colorectal, breast, cervical, squamous cell carcinoma, un-differentiated/ poorly differentiated carcinoma, cholangio-carcinoma, oesophageal adenocarcinoma, fibrosarcoma	iv	1×10^{10} $TCID_{50}$ once every 3 weeks
REO 010	I	C	23	Esophagus, prostate, melanoma, pancreas, unknown primary, breast, stomach, mesothelioma, hepatocellular, bronchoalveolar	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 3 weeks
REO 011	I/II	C	31	Squamous cell head and neck, head and neck, melanoma, gynecological, sarcoma, unknown primary	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 3 weeks
REO 012	I	O	36	Advanced or metastatic solid tumors including pancreatic, lung, ovarian	iv	Escalating dose
OSU-07022 (NCI)	I/II	O	70	Metastatic ovarian epithelial, primary peritoneal, fallopian tube	iv/ip	iv: 3×10^{10} $TCID_{50}$ 5 consecutive days every 3 weeks IP: 3×10^{9} $TCID_{50}$ 5 on days 2 and 3 of 3 week cycle
MAYO- MC0672 (NCI)	II	C	21	Visceral, soft tissue, and osseous metastatic melanoma	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 4 weeks
REO 013	Translational	C	10	Metastatic colorectal cancer to the liver	iv	1×10^{10} $TCID_{50}$ 5 consecutive days for one cycle

Dose of combination therapeutic	Response	Toxicities		Reference(s)
		Grade I/II	Grade III/IV	
N/A	(OR) 1 CR 2 PR 4 SD 10 PD	Nausea, vomiting, headache, local erythema, fever/chills, diarrhea, dizziness, athralgia/myalgia, flu-like illness, ALT increase, lymphopenia	Headache, lymphopenia	58
Surgery	NR	Flu-like illness	N/A	45
N/A	(BR) 1 SD 10 PD	Fever, headache, nausea, vomiting, anorexia, weight loss	GGT elevation	48
N/A	(BR) 1 PR 7 SD 10 PD	Fever, chills, myalgia, headache, sore throat/nasal fullness, fatigue, nausea, diarrhea, anorexia	N/A	59
N/A	(OR) 8 SD 2 PD	Flu-like illness, fever, chills, fatigue, headache, nausea, vomiting, hyperhidrosis, lymphopenia, neutropenia, thrombocytopenia	Lymphopenia, neutropenia	60
Palliative radiation Phase Ia: 20 Gy in 5 fractions; Phase Ib: 36 Gy in 12 fractions	(OR) 7 PR 7 SD	Fever, flu-like illness, fatigue, nausea, vomiting, chills, lymphopenia, neutropenia	Lymphopenia	68
N/A	(BR) (OR) 1 PR 1 PR 10 SD 2 SD 4 PD 12 PD	Convulsions, aphasia, hyperglycemia	Convulsions	49
Low-dose radiation 20 Gy in 5 consecutive fractions	(OR) 4 PR 2 MnR 7 SD	NR	NR	69
Gemcitabine 1,000 mg/m^2 twice every 3 weeks	(OR) 1 PR 7 SD	Fever, nausea, vomiting, diarrhea, chills, ALT increase, neutropenia	ALT and AST increase, neutropenia, troponin I rise	87
Docetaxel 75 mg/m^2 3 times weekly	(OR) 4 PR 3 MnR 7 SD			75
Paclitaxel 175 mg/m^2 and carboplatin AUC 5 once every 3 weeks	(OR) 1 CR 6 PR 2 MjR 9 SD 8 PD	Blood cytopenias, nausea, vomiting, fatigue, diarrhea, infection, hair loss, muscle pain, fever, chills, flu-like illness	Neutropenia, lymphopenia, anemia, fever, myalgia, diarrhea, nausea, vomiting, hypotension	66
Cyclophosphamide	NYR	NYR	NYR	71
N/A	NYR	NYR	NYR	55
N/A	(OR) 6 SD	Fatigue, nausea, fever, anemia, chills, myalgia, anorexia, anemia, vomiting, diarrhea	Fatigue, lymphopenia, hyponatremia	61
Surgery	NR	Flu-like illness, nausea, constipation, headache, fever, myalgia, pyrexia, rogors, leucopenia	N/A	94

(*Continued*)

Table 1 (*Continued*)

Trial number[a]	Phase	Status	No of patients[b]	Cancer type(s)	Mode of administration	Dose of reovirus[c]
REO 013 brain	I	O	NR	Recurrent malignant glioma	iv	Escalating dose once
REO 014	II	C	52	Synovial sarcoma, leiomyosarcoma, osteosarcoma, malignant fibrous histiocytoma and other sarcomas metastatic to the lung	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 4 weeks
REO 015	II	C	14	Platinum-refractory recurrent and/or metastatic squamous cell cancers of the oral cavity, larynx, or pharynx	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 3 weeks
REO 016	II	O	36	NSCLC with K-Ras or EGFR-activated tumors	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 3 weeks
REO 017	II	O	33	Advanced or metastatic pancreatic adenocarcinoma	iv	1×10^{10} $TCID_{50}$ on days 1, 2, 8, and 9 every 3 weeks
REO 018	III	O	280	Platinum-refractory recurrent and/or metastatic head and neck squamous cell carcinoma of the, or nasopharynx squamous cell cancer	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 3 weeks
REO 020	II	O	43	Metastatic malignant melanoma	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 3 weeks
REO 021	II	O	55	Advanced stage or recurrent lung squamous cell carcinoma	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 3 weeks
REO 022	I	R	21	K-Ras mutant metastatic colorectal	iv	Escalating dose 5 consecutive days every 4 weeks
GOG-0186H (NCI)	II	R	110	Recurrent or persistent ovarian epithelial, fallopian tube, or primary peritoneal cancer	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 4 weeks
COG-ADVL1014 (NCI)	I	O	45	Pediatric refractory or relapsed solid tumors	iv	Escalating dose 5 consecutive days every 4 weeks
OSU-10045 (NCI)	II	R	70	Recurrent or metastatic pancreatic cancer	iv	3×10^{10} $TCID_{50}$ 5 consecutive days every 3 weeks
OSU-11148 (NCI)	I	O	12	Refractory multiple myeloma	iv	Escalating dose 5 consecutive days every 4 weeks
IND 209 (NCIC)	II	R	80	Recurrent or metastatic castration resistant prostate cancer	iv	5 consecutive days every 3 weeks (exact dose NR)
IND 210 (NCIC)	II	R	100	Metastatic colorectal adenocarcinoma	iv	5 consecutive days every 4 weeks (exact dose NR)
IND 211 (NCIC)	II	R	150	Previously treated advanced or metastatic NSCLC	iv	4.5×10^{10} $TCID_{50}$ 3 consecutive days every 3 weeks

Dose of combination therapeutic	Response	Toxicities		Reference(s)
		Grade I/II	Grade III/IV	
Surgery	NYR	NYR	NYR	63
N/A	(OR) 19 SD 25 PD	Flu-like illness, fever, fatigue, myalgia, cough, congestion, neutropenia, AST/ALT increase, diarrhea	Neutropenia	62,96,97
Paclitaxel 175 mg/m^2 and carboplatin AUC 5 once every 3 weeks	(OR) 4 PR 2 SD	NR	NR	51,52
Paclitaxel 175 mg/m^2 and carboplatin AUC 5 once every 3 weeks	NYR	NYR	NYR	98,99
Gemcitabine 175 mg/m^2 on days 1 and 8 every 3 weeks	(So far) 1 PR 12 SD 5 PD	(So far) asthenia, fever, flu-like illness, nausea, vomiting	(So far) neutropenia, fever	72–74
Paclitaxel 175 mg/m^2 and carboplatin AUC 5 once every 3 weeks	NYR	(So far) Mild fever, chills, nausea, diarrhea	NYR	53,54
Paclitaxel 200 mg/m^2 and carboplatin AUC 6 once every 3 weeks	(So far) 3 PR 7 SD	NYR	NYR	76,100
Paclitaxel 200 mg/m^2 and carboplatin AUC 6 once every 3 weeks	(So far) (BR) (OR) 10 PR 10 PR 14 SD 12 SD 1 PD 3 PD	NYR	NYR	75,101
Irinotecan 125–180 mg/m^2, Leucovorin 400 mg/m^2, Fluorouracil 400 mg/m^2 bolus +2400 mg/m^2, and Bevacizumab 5 mg/kg once every 2 weeks	NYR	NYR	NYR	79,80
Paclitaxel on days 1, 8, and 15 every 4 weeks (exact dose NR)	NYR	NYR	NYR	56,57
Cyclophosphamide orally for 21 consecutive days every 4 weeks (exact dose NR)	NYR	NYR	NYR	77,78
Paclitaxel and carboplatin once every 3 weeks (exact dose NR)	NYR	NYR	NYR	102,103
N/A	NYR	NYR	NYR	64,65
Docetaxel 75 mg/m^2 once every 3 weeks and prednisone 5 mg twice daily	NYR	NYR	NYR	46,47
FOLFOX-6 and bevacizumab every 2 weeks (exact dose NR)	NYR	NYR	NYR	83,84
Pemetrexed: 500 mg/m^2 once every 3 weeks or docetaxel 75 mg/m^2 once every 3 weeks	NYR	NYR	NYR	85,86

(*Continued*)

Table 1 (*Continued*)

Trial number[a]	Phase	Status	No of patients[b]	Cancer type(s)	Mode of administration	Dose of reovirus[c]
IND 213 (NCIC)	II	R	100	Advanced or metastatic breast cancer	iv	Days 1, 2, 8, 9, 15 and 16 every 4 weeks (exact dose NR)

Notes: [a]Trail number as reported by Oncolytics Biotech® Inc., Calgary, AB, Canada; [b]no of patients – for completed trials, reported number is the number of patients that were treated (does not include those that were enrolled but not treated). For ongoing and actively recruiting trials, number is reported estimated number of patients who will enroll in the trial; [c]reovirus dose: maximum dose attempted is reported with the exception of the REO 009 trial in which the indicated dose is the highest dose permitted based on toxicity findings.

Abbreviations: N/A, Not Applicable; AUC, Area under curve; NYR, Not Yet Reported; C, completed; O, ongoing; R, actively recruiting; CR, complete response; PR, partial response; MjR, major response; MnR, minor response; SD, stable disease; PD, progressive disease; NR, not reported; OR, overall response; BR, best response; $TCID_{50}$, tissue culture infectious dose 50; NSCLC, non-small-cell lung carcinoma; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; EGFR, epidermal growth factor receptor; NCI, National Cancer Institute; FOLFOX-6, fluorouracil plus leucovorin and oxaliplatin; AUC, area under the curve; iv, intravenous; ip, intraperitoneal; ITu, intratumoral.

malignancies and their associated metastases. In the first study, of the 16 of 18 patients that were evaluable for the objective response by imaging studies as determined by RECIST, one patient showed a PR with a 34% shrinkage in tumor burden, and seven patients demonstrated SD irrespective of dose level.[59] Histological examination of the posttreatment biopsy samples showed extensive necrosis that was accompanied by markers of viral replication and remnant viral particles. Similarly, the second study treated 33 patients, three of whom had reduced levels of tumor markers (carcinoembryonic antigen, prostate-specific antigen, and carbohydrate antigen 19.9) following reovirus administration.[60] Computed tomography also revealed evidence of tumor necrosis in one patient, quantified as a 12% reduction, and biopsy analysis confirmed viable virus in three patients where recovered reovirus titer directly correlated with the dosage administered. Taken together, the results of these two Phase I trials using systemically administered reovirus supported the previously reported antitumor activities of reovirus, and led to the conclusion that monthly intravenous injections of reovirus are safe and well tolerated in patients with advanced malignancies.

The first Phase II clinical trial for intravenous reovirus monotherapy began enrollment in 2008 for the treatment of metastatic melanoma.[61] Twenty-one patients were injected intravenously with reovirus at a dose of 3×10^{10} 50% tissue-culture infective dose ($TCID_{50}$) once every 60 minutes on days 1–5 every 4 weeks, and were monitored for clinical benefits of CR or PR for at least 8 weeks. Although no patients met the criteria for CR or PR, one patient did demonstrate extensive (75%–90%) tumor necrosis in two metastatic lesions following two treatment cycles. Additionally, productive reovirus replication was detected in two of 13 biopsies that contained melanoma metastases and were performed 1 week following treatment, further supporting the positive clinical outcomes from the earlier Phase I results.[59,60] Additional evidence was gathered for the safety of reovirus as there were few severe (grade III or IV) toxicities reported. Furthermore, two of 13 patients demonstrated productive reoviral replication in melanoma metastases; however, the study's clinical objective to achieve at least two or more patients having a CR or PR was not met, and thus the trial could not proceed as planned. Therefore, this Phase II trial did not support the use of reovirus as a monotherapy for metastatic melanoma, but the evidence gathered suggested that reovirus may be more efficacious as part of a combination therapy with other chemotherapeutic agents. In contrast, another Phase II clinical trial for intravenous administration of reovirus for 52 patients with lung metastases of soft-tissue sarcomas demonstrated a total clinical benefit rate of 43% (ie, 19 of 44 evaluable patients experienced stable disease for as short as 2 months but as long as 22 months).[62] Positive results from this completed trial showed promise for intravenous reovirus therapy for metastatic sarcomas; however, like the aforementioned Phase II trial, they suggested that a combination of reovirus with other therapeutic options could greatly enhance reovirus therapy and promote better patient outcomes.

There are three ongoing clinical trials for reovirus as a monotherapy. The first began in 2008, and is a Phase I/II combined clinical trial conducted in partnership with the US National Cancer Institute for intravenous and intraperitoneal administration of reovirus for patients with metastatic ovarian, peritoneal, and fallopian tube cancers that have not responded to platinum chemotherapy.[55] Thus far, the study has concluded that there is evidence of selective reovirus penetration in peritoneal tumors, and no DLTs had been observed as of 2010. The second ongoing trial, performed by the University of Leeds in the UK, is a Phase I trial in which the intravenous administration of reovirus is done prior to surgery in patients with recurrent high-grade primary or metastatic brain tumors.[63] The third trial is a Phase I clinical trial for intravenous administration of reovirus for patients with relapsed multiple myeloma, and has an estimated primary completion date of December 2014.[64,65] This is the first

Dose of combination therapeutic	Response	Toxicities		Reference(s)
		Grade I/II	Grade III/IV	
Paclitaxel on days 1, 8, and 15 every 4 weeks	NYR	NYR	NYR	81,82

clinical trial to examine the use of reovirus as a treatment for hematological malignancies.

Thus far, the most frequently reported toxicities from reovirus have been low-grade (I or II) flu-like symptoms. Adverse events due to reovirus include nausea, vomiting, headache, fever/chills, diarrhea, anorexia, and weight loss; however, all appear to be independent of treatment dose or cycle. Two studies using intratumoral administration of reovirus showed no severe toxicities at all associated with reovirus.[45,48] Most toxicities, when observed, occur early in the treatment regimen and were better tolerated in the later cycles. Some studies show hematological abnormalities, including lymphopenia and neutropenia often of grade III or IV; however, these occurrences were few and were usually unlikely to be related to reovirus, and typically did not result in negative clinical consequences.[58,60,66] In contrast, other studies showed no evidence of hematological toxicities at all.[59,67] Taken together, toxicity results indicate that reovirus is well tolerated by patients, with generally only mild manageable adverse events.

Combination therapy with reovirus in clinical studies

Both preclinical and clinical studies thus far have demonstrated a higher efficacy for reovirus cancer therapy when reovirus is combined with other forms of cancer therapy, including radiotherapy and single- and multiple-chemotherapy regimens. Not surprisingly, the majority of ongoing clinical trials involve the administration of reovirus in combination with an additional anticancer therapeutic agent.

The first combination reovirus clinical trial treated patients with advanced cancers, including one of unknown primary origin, melanoma, head and neck cancer, squamous cell carcinoma of the skin, lung, ovarian, colorectal, esophageal, and pancreatic cancer in a two-stage Phase I dose-escalation study using intratumoral reovirus and palliative radiotherapy.[68] Here, reovirus doses ranging from 1×10^8 to 1×10^{10} $TCID_{50}$ and radiation levels of 20–36 Gy were used in combinations. This study concluded that reovirus antitumor activity is unaffected by high doses of radiation, and that the combination of the two is safe for further investigations. Following the initial study, a subsequent Phase II trial[69] was performed using low-dose radiation, and demonstrated a 93% total disease-control rate (combined CR, PR, and SD) in treated lesions, thereby confirming the safety and positive clinical outcomes of the combination regimen. As opposed to palliative radiotherapy, it is expected that reovirus will be used in future trials in combination with radical radiotherapy for a curative intent.

The first chemotherapeutic agent used in combination with reovirus was gemcitabine for the treatment of 16 patients with advanced malignancies, including squamous cell, undifferentiated, and poorly differentiated carcinoma, cholangiocarcinoma, esophageal adenocarcinoma, fibrosarcoma, NSCLC, and colorectal, breast, and cervical cancer.[70] Through this study, the concluding recommended dose for this particular combination was as follows: reovirus 1×10^{10} $TCID_{50}$ on day 1 and gemcitabine at 1,000 mg/m^2 on day 1 and day 8 of a 21-day cycle. Of the ten evaluable patients, one had a PR and a minor decrease in tumor size after just four cycles of therapy. Although this combination was determined to be safe enough for further studies, this trial highlighted the importance of potential safety issues when combining reovirus with immunomodulatory agents that enhance antitumor immune responses. This study cautions that a fine balance must be achieved between agents that dampen antiviral immune responses, as they could potentially exacerbate reovirus toxicities and pose a minor but real threat to patient safety. For instance, the ongoing Phase I clinical trial using cyclophosphamide, a known immunosuppressant drug, in combination with reovirus (REO 012) includes a primary objective of determining the minimum effective immunomodulatory dose of cyclophosphamide.[71] Here, it will be important to monitor the effect of cyclophosphamide on

antiviral immune responses and the potential for exacerbation of reovirus toxicities. A Phase II clinical trial is ongoing to further evaluate the interactions between reovirus and gemcitabine in a clinical setting.[72–74]

Other chemotherapeutic agents that have completed at least Phase I clinical trials in combination with reovirus include docetaxel,[75] and paclitaxel and carboplatin.[66] All aforementioned combinations have gone on to further Phase II or Phase III clinical trials. In either monotherapy or combination therapy studies, the recommended dose of reovirus is 3×10^{10} $TCID_{50}$ once over 60 minutes on days 1–5 every 3 or 4 weeks. The recommended dose of docetaxel in combination therapy is 75 mg/m^2 three times weekly.[46,47,75] As for paclitaxel and carboplatin, paclitaxel is recommended at a dose of 175 mg/m^2, and carboplatin at an area under the curve (AUC) of 5[51,52,66,76] or paclitaxel at 200 mg/m^2 and carboplatin at AUC 6[76,77] on day 1 of a 3-week cycle. Other chemotherapeutic agents currently in or recruiting patients for Phase I trials with reovirus include cyclophosphamide,[71,78,79] FOLFIRI (folinic acid, fluorouracil, irinotecan)/bevacizumab,[80,81] paclitaxel alone[82,83], FOLFOX (folinic acid, fluorouracil, oxaliplatin)-6/bevacizumab,[84,85] docetaxel and prednisone,[46,47] and docetaxel and pemetrexed.[86,87]

Overall, combination therapies have demonstrated that reovirus does not exacerbate known chemotherapy or radiation-associated toxicities or vice versa.[66,68,75] However, the known toxicities of gemcitabine and reovirus were observed in one trial.[70] Lolkema et al concluded that since gemcitabine appears to have an inhibitory effect on the development of neutralizing antireoviral antibodies (NARAs), a chemotherapeutic regimen in combination with reovirus may exacerbate reovirus toxicity in the liver and further elevate liver enzyme levels.[70]

Key obstacles to reovirus oncotherapy

With a total of 32 clinical trials (completed or ongoing) and continued progress in preclinical studies, reovirus therapy is well set to transition from "bench" to "bedside" for the treatment of a multitude of cancers. At the same time, these studies have also recognized key obstacles that will in future dictate the successful translation of reovirus oncotherapy into clinical practice. These key obstacles are associated with antiviral immune responses and delivery of reovirus to cancer cells.

Host immune responses pose the biggest challenge for reovirus oncotherapy. Reovirus-induced immunological events in tumor-bearing hosts are very complex and unique, as they involve the opposing and concurrent effects of antiviral and antitumor immunity. Type I IFNs, produced immediately following the exposure of host to reovirus, can directly inhibit virus replication.[27,88] Furthermore, such IFNs in combination with other proinflammatory cytokines (ie, TNFα, IL-1, IL-6, IL-12, etc) augment the innate immune response through the recruitment and activation of immune cells, such as neutrophils, macrophages, DCs, and NK cells,[89] and hamper reoviral spread. Similarly, antiviral adaptive immunity, wherein T cells contain viral replication via direct killing of virally infected cells or by the production of cytokines, such as IFNγ[90] and B cells, produce NARAs and curtail the replication and spread of reovirus prematurely before it can eliminate all the cancer cells. It should be noted that NARAs are prevalent in most adults, owing to exposure to reovirus sometime during their lifetime.[45,59–61,66,68,70,75,91,92] Therefore, future directions need to focus on specifically targeting the antiviral immune response, such as T-cell and B cell responses, without affecting the production of strong antitumor immunity.

The other related challenge centers on the delivery of reovirus to tumor cells. Although intratumoral injections are designed to deliver all of the virus particles to the tumor vicinity, systemic viral administration has several advantages. In particular, systemic delivery increases the probability that the virus can reach metastatic tumors or tumors that constitute multiple nodules that are not confined to a particular area. Unfortunately, various host factors, programmed to restrict the spread of pathogens, such as NARAs, nonspecific uptake by organs (spleen, lung, and liver) and scavenging immune cells, and poor virus escape from the vascular compartment,[93,94] significantly affect virus delivery through systemic routes. As reviewed by Roy et al, multiple steps have been attempted to ameliorate the systemic delivery of OVs with the use of cell carriers.[95] It is noteworthy that immune cells loaded with reovirus can shield the virus from circulating NARAs and deliver it to tumor cells,[92,96] and represent a clinically feasible means of enhancing systemic reovirus delivery to the tumor cells.

Conclusion and future directions

Reovirus oncotherapy has shown definitive promise through preclinical and clinical trials, and is en route to becoming a clinically practiced therapeutic option for the treatment of various cancers. In this review, we hope to have clearly outlined the progress that has been made in reovirus clinical trials, from monotherapies to combination trials, by providing up-to-date trial results from completed, ongoing,

and upcoming studies. In future, a major thrust of preclinical research should be on overcoming the current obstacles to reovirus oncotherapy, and translating that knowledge to clinical trials.

Considering the focus of this article, we have not addressed the immunological aspects of reovirus oncotherapy. As is being recognized for all OVs, host immune responses play contrasting roles during reovirus oncotherapy. On one hand, antiviral immune responses hamper the efficacy of oncotherapy. On the other hand, antitumor immunity further potentiates anticancer effects. It is clear that research into oncotherapy-driven immune responses is a major priority, as the strategic management of antiviral and antitumor immune responses holds the key to achieving optimum efficacy of reovirus oncotherapy.

Disclosure

The authors report no conflicts of interest in this work.

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