

A Phase I Study of the Combination of Intravenous Reovirus Type 3 Dearing and Gemcitabine in Patients with Advanced Cancer

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Abstract

Purpose: This study combined systemic administration of the oncolytic reovirus type 3 Dearing (reovirus) with chemotherapy in human subjects. We aimed to determine the safety and feasibility of combining reovirus administration with gemcitabine and to describe the effects of gemcitabine on the antireoviral immune response.

Experimental Design: Patients received reovirus in various doses, initially we dosed for five consecutive days but this was poorly tolerated. We amended the protocol to administer a single dose and administered up to 3×10^{10} TCID₅₀. Toxicity was assessed by monitoring of clinical and laboratory measurements. We assessed antibody response by cytotoxicity neutralization assay.

Results: Sixteen patients received 47 cycles of reovirus. The two initial patients and one patient in the final cohort experienced dose limiting toxicity (DLT). The DLTs consisted of two asymptomatic grade 3 liver enzyme rises and one asymptomatic grade 3 troponin I rise. Common toxicities consisted of known reovirus and gemcitabine associated side effects. Further analysis showed a potential interaction between reovirus and gemcitabine in causing liver enzyme rises. Grade 3 rises in liver enzymes were associated with concomitant aminocetophen use. Importantly, the duration of the liver enzyme rise was short and reversible. Neutralizing antibody responses to reovirus were attenuated both in time-to-occurrence and peak height of the response.

Conclusions: Reovirus at the dose of 1×10^{10} TCID₅₀ can be safely combined with full dose gemcitabine. Combination of reovirus with gemcitabine affects the neutralizing antibody response and this could impact both safety and efficacy of this treatment schedule. *Clin Cancer Res*; 17(3); 581–8. ©2010 AACR.

Introduction

Reovirus type 3 Dearing (reovirus; REOLYSIN; Oncolytics Biotech Inc) is a wild-type member of the Reoviridae family and is nonpathogenic in humans. Infections are usually asymptomatic, however, reovirus infection of cancer cells results in specific cytolysis. Activated signalling

pathways downstream of *KRAS* or *EGFR* suppress the activity of double stranded RNA activated protein kinase (PKR), which normally inactivates viral replication (1,2). Therefore, reovirus is being tested as an anticancer therapy targeted at *KRAS* mutant tumors.

Systemic administration of reovirus has been tested in human subjects in 2 phase I monotherapy trials. The first trial used a single infusion of 1×10^8 to 3×10^{10} tissue culture infective dose (TCID)₅₀ every 4 weeks and the second trial tested treatment schedule infusing up to 3×10^{10} TCID₅₀ 5 consecutive days every 4 weeks (3,4). Neither study observed dose limiting toxicities (DLT). Commonly observed mild toxicities included fever, fatigue and headache. Dose escalation stopped at 3×10^{10} TCID₅₀ because of limited quantities of reovirus available for clinical use at that time. Importantly, both studies showed evidence of viral replication in tumor tissue samples taken after treatment, indicating that systemic treatment with reovirus delivers the virus to the tumor site. The viral shedding after systemic delivery was minimal. The conclusion from these studies is that intravenous single agent reovirus administration is safe.

This trial studied the combination of reovirus with gemcitabine. Exploration of combinations of reovirus with chemotherapy in clinical trials is an important step towards

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Translational Relevance

Reovirus type 3 Dearing (reovirus) is a potential anticancer therapy that targets tumor types with activating mutations in the *KRAS* oncogene or *EGFR* overexpression. Recent evidence suggests that reovirus can safely be delivered systemically. This is the first study to report on a combination of reovirus with chemotherapy. The combination of reovirus with gemcitabine is safe however gemcitabine does affect the neutralizing antibody response against reovirus. This study in human subjects validates the preclinical data on chemotherapy-induced attenuation of neutralizing antibody responses. The development of combination therapies using oncolytic viruses is essential to unlock their full potential and our data indicate that immune monitoring is an essential feature of trials that explore such combinations.

unlocking the full potential of reoviral therapy (5–7). The combination of systemic reovirus administration with concomitant chemotherapy, including gemcitabine has shown synergy in various *in vivo* model systems (6–8). Lung and pancreatic cancer are tumor types that harbor common mutations in the *KRAS* oncogene and should be targeted by reovirus treatment. For both these diseases gemcitabine either as combination or single agent is standard of care treatment. *In vivo* testing of the combination of reovirus and gemcitabine in mice showed remarkable antitumor effects with pathologic complete remissions in 4/5 mice (9). These data pose a strong rationale to combine reovirus and gemcitabine.

The combination of reovirus-based oncolytic therapy with chemotherapy is not expected to result in overlapping toxicity based on the known toxicity profiles; however chemotherapy may blunt an immune response to viral antigens and thereby may increase the exposure and toxicity of the virus to normal tissues (5). Exposure to reovirus results in the induction of neutralizing antireovirus antibodies (NARA) and baseline NARA levels can be detected in up to 100% of healthy human adults (5). The immunological response to reovirus infusion has been documented in Phase I studies and lead to a fast and effective increase in NARA levels (10). The coadministration of full dose cyclophosphamide with reovirus in mice resulted in marked tumor regression, however it also induced lethal cardiac toxicity (11). Reoviral replication could be detected in cardiac cells due to complete suppression of the NARA response and led to a massive myocarditis. Lower doses of cyclophosphamide were well tolerated and blunting of the NARA response did lead to better reovirus delivery to the tumor. A number of reports have shown that immunosuppression given concomitantly with reovirus treatment in mice, using low dose cyclophosphamide, cyclosporine, or antibodies to CD4/CD8 improves treatment efficacy (8,12). This trial is the first study to report a combination of reovirus with

chemotherapy in humans and we included NARA measurements to document any changes that could contribute to toxicity and potential efficacy.

Here we report the results of a Phase I, open-label, dose-escalation study of the combination of reovirus and gemcitabine that aimed to determine the safety and tolerability of systemic reovirus when coadministered with gemcitabine treatment and thus determine the MTD for reovirus in this combination. Secondary endpoints included the characterization of the immune response to reovirus given with gemcitabine; to evaluate the pharmacokinetics of gemcitabine when combined with reovirus; and to describe any antitumor activity.

Methods

Patients and eligibility criteria

This was a nonrandomised, open-label, phase 1, dose-escalation study conducted at 2 participating institutions. The study was conducted in accordance with the International Conference on Harmonisation Good Clinical Practice with the ethical principles of the current Declaration of Helsinki and was approved by a UK Central Research Ethics Committee. All patients provided written informed consent before any study-related procedures were performed. All patients had to fulfill all selection criteria (Supplemental Methods).

Treatment administration

Screening was performed according to protocol (Supplemental Methods) The study was designed to determine the MTD for reovirus combined with gemcitabine administered at a dose of 1,000 mg/m² on days 1 and 8 of a 21-day cycle. Gemcitabine was reconstituted according to the manufacturer's instructions and was administered as a 30-minute intravenous infusion prior to reovirus administration. Reovirus (REOLYSIN) was supplied by Oncolytics Biotech Inc and administered according to protocol (Supplemental Methods).

The starting dose of reovirus was 3×10^9 TCID₅₀ administered on days 1 to 5 of each cycle (cohort 1). However, the first 2 patients that were treated at this dose level experienced a DLT as defined in the protocol. The schedule of reovirus administration was subsequently amended so that it was only administered on day 1 of each treatment cycle. Patients were treated at 1 of 4 predetermined escalating dose levels of 1×10^9 (cohort 2), 3×10^9 (cohort 3), 1×10^{10} (cohort 4), and 3×10^{10} TCID₅₀ (cohort 5). Study treatment was continued until there was evidence of disease progression, unacceptable toxicity despite dose modification, or until the patient or investigator decided to discontinue study treatment.

Toxicity assessments

Toxicities were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 and safety assessments were performed according to protocol (Supplementary Methods) DLT was defined as one of the

following treatment-related events occurring during the first cycle of treatment: grade 4 neutropenia lasting for more ≥ 7 days; grade 4 neutropenia with sepsis; platelet count $< 0.25 \times 10^9/L$; grade 2 neurotoxicity or cardiotoxicity; inability to tolerate 1 cycle of treatment due to toxicity; any other drug-related nonhematologic grade 3 or 4 toxicity except flu-like symptoms and nausea and vomiting if appropriate prophylactic or therapeutic measures had not been administered.

If 1 out of 3 patients experienced a DLT during the first treatment cycle, up to 3 additional patients were treated at that dose level. The MTD was defined as the highest dose level at which fewer than 2 out of 6 patients experienced a DLT.

The protocol defined rules for dose reductions and re-treatment criteria (Supplemental Methods).

Response assessments

Disease assessments were performed at pretreatment and after every 2 cycles of therapy by CT scanning of measurable and assessable disease sites. Response was determined by RECIST (13). Disease assessments also included tumor markers (carcinoembryonic antigen [CEA], prostate specific antigen [PSA], CA125, and CA19.9) where appropriate.

Gemcitabine pharmacokinetics (PK)

Blood samples were taken during the first cycle of treatment at the following time points: before gemcitabine, 15 and 30 minutes after gemcitabine, 90 minutes after gemcitabine, directly after the reovirus infusion, 4, 6, 8, 24 and 48 hours. Concentrations of gemcitabine were quantified in plasma using an LC-MS/MS method, with a lower limit of quantification of 5 ng/mL according to published methodology and compared to historic data (14–16). PK parameters were estimated for each patient using a fully validated version of WinNonlin Pro (Version 5.2.1; Pharsight Corporation Inc., 2008)

Analysis of viral shedding by reverse transcription PCR and detection of neutralizing antireoviral antibodies

Methods for analysis of viral shedding and NARA were performed as previously described (10). Samples (blood, urine, stool, and sputum) for reovirus shedding were taken each cycle before start of treatment, 4 hours after treatment and at day 15. If the day 15 sample was negative for virus, subsequent pretreatment testing was omitted. NARA levels were determined weekly for the first 2 cycles of treatment. Data are given as the dilution of serum that gave $> 80\%$ L929 cell kill in a standardized assay.

Results

Patients

Sixteen patients with a variety of solid malignancies were enrolled and treated over 5 dose levels with a total of 47 cycles administered. Patient demographics are displayed in Table 1.

Table 1. Patient demographics, tumor diagnoses, performance status, and prior therapies at baseline

Patient characteristics	No. patients
Total no.	16
Age (years)	
Median in different dose levels	50.2–59.8
Range	36–72
Male/female	10/6
Primary tumor site	
NSCLC	1
Colorectal	3
Breast	1
Cervical	2
Squamous cell carcinoma	3
Undifferentiated carcinoma	1
Poorly differentiated carcinoma	1
Cholangiocarcinoma	1
Oesophageal adenocarcinoma	2
Fibrosarcoma	1
Eastern Cooperative Oncology Group performance status	
0	2
1	13
2	0
No. of prior chemotherapy regimens	
0	0
1	5
2	4
≥ 3	9
No. prior immunotherapy regimens	
0	16
1	0
No. prior investigational agents	
0	13
1	2
2	1
Prior radiation therapy	13
Prior surgery	15

Dose escalation and dose limiting toxicity

Dose escalation started with reovirus infusion on days 1 to 5 of each cycle. Only 2 patients were recruited to this cohort. The first patient experienced a DLT due to a transient and reversible grade 3 rise in liver enzymes (ALT and AST). Although we could not allocate definitive causality, the rise in liver enzymes was considered probably due to gemcitabine treatment. Gemcitabine is known to cause a rise in liver enzymes and due to the temporal association a relationship was deemed probable. However, we cannot exclude the possibility that this event was caused by either reovirus or metastatic disease. The second patient experienced a DLT due to an asymptomatic grade 3 Troponin I rise after the second dose of reovirus. Further cardiac evaluation (ECG, echocardiogram) did not reveal any other

Table 2. Incidence of reovirus related adverse events reported in at least 10% of patients overall safety population

	Dose group (TCID ₅₀) with gemcitabine (1,000 mg/m ²)				
	3 × 10 ⁹ (days 1–5) N = 2	1 × 10 ⁹ (day 1) N = 3	3 × 10 ⁹ (day 1) N = 4	1 × 10 ¹⁰ (day 1) N = 4	3 × 10 ¹⁰ (day 1) N = 3
Number of patients with at least one AE*	2 (100)	3 (100)	4 (100)	4 (100)	3 (100)
Pyrexia	0	2 (66.7)	4 (100)	2 (50.0)	3 (100)
Nausea	1 (50.0)	2 (66.7)	1 (25.0)	2 (50.0)	1 (33.3)
Vomiting	2 (100)	1 (33.3)	1 (25.0)	2 (50.0)	0
Diarrhoea	0	2 (66.7)	2 (50.0)	2 (50.0)	0
Chills	0	1 (33.3)	1 (25.0)	1 (25.0)	2 (66.7)
ALT increased	1 (50.0)	1 (33.3)	1 (25.0)	1 (25.0)	1 (33.3)
Neutropenia [†]	0	2 (66.7)	2 (50.0)	0	0
AST increased	1 (50.0)	0	1 (25.0)	1 (25.0)	1 (33.3)
Fatigue	0	0	0	2 (50.0)	1 (33.3)
GGT increased	1 (50.0)	1 (33.3)	1 (25.0)	0	0
Lethargy	0	1 (33.3)	1 (25.0)	1 (25.0)	0
Rash	0	2 (66.7)	1 (25.0)	0	0
Febrile neutropenia	0	1 (33.3)	1 (25.0)	0	0
Thrombocytopenia	0	0	0	1 (25.0)	1 (33.3)
Dry mouth	0	1 (33.3)	0	1 (25.0)	0
Blood alkaline phosphatase increased	1 (50.0)	1 (33.3)	0	0	0
Troponin I increased	1 (50.0)	1 (33.3)	0	0	0
Anorexia	0	1 (33.3)	0	0	1 (33.3)
Myalgia	0	1 (33.3)	0	0	1 (33.3)
Alopecia	0	1 (33.3)	1 (25.0)	0	0
Pruritus	0	1 (33.3)	1 (25.0)	0	0

Abbreviations: AEs = adverse events; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; TCID₅₀ = tissue culture infective dose₅₀.

*Number of patients with an event (percentage of the total).

[†]Includes term White blood cell count decreased.

indication for cardiac abnormalities. The temporal relation suggests a causal relation between this event and the reovirus/gemcitabine treatment. As 2/2 patients experienced a DLT in the first cohort, the protocol was amended and the dose of reovirus was lowered to 1 × 10⁹ TCID₅₀ on day 1 of each cycle. The subsequent cohorts with reovirus treatment only on day 1 did show one more DLT at the dose level of 3 × 10¹⁰ TCID₅₀. This patient had a grade 3 rise in liver enzymes (ALT) which occurred during the first cycle. This patient was re-challenged with gemcitabine alone in the second cycle and did not develop liver enzyme abnormalities in this cycle; therefore this event was deemed related to the combination of reovirus and gemcitabine.

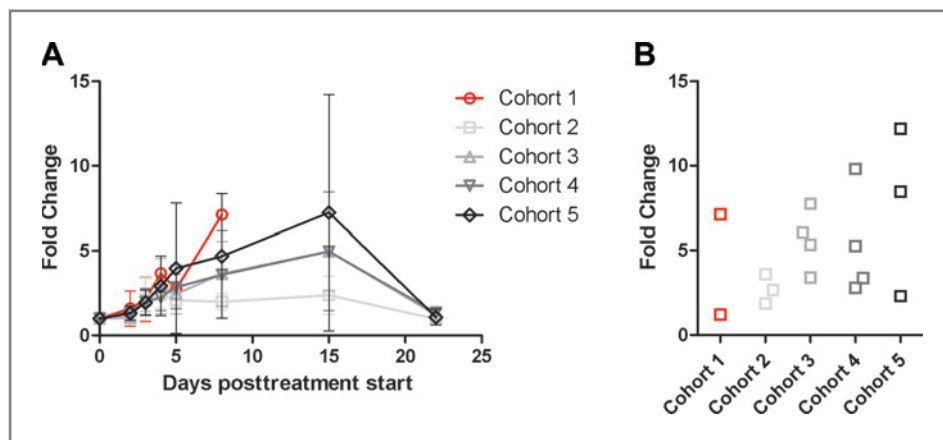
Safety

Treatment related adverse events for reovirus were similar to those observed in the previous 2 Phase I studies. The

treatment related toxicities that occurred in more than 10% of patients are given in Table 2. The most frequently reported treatment-related adverse events were pyrexia (68.8%), nausea (43.8%), diarrhea (37.5%), vomiting (37.5%), chills (31.3%), and increased ALT (31.3%). The adverse events were generally mild or moderate in severity and manageable without reovirus dose reduction or discontinuation. Importantly, the expected gemcitabine related side effects: rise in liver enzymes (ALT and AST) occurred in 5/16 patients with a grade 3 rise in 2/5 patients and grade 3 or more neutropenia occurred in 4/16 patients. These numbers are comparable to the prescription information on gemcitabine (17).

As 2/3 DLTs were related to liver enzyme rises and had not been observed in the single agent phase I trials, we analyzed the data on liver enzyme changes more carefully. Of note, both patients with grade 3 liver enzyme elevations had concomitant antipyretic medication of acetaminophen

Figure 1. Relative ALT levels during the first cycle of treatment: (A) Relative levels of ALT are plotted. All values have been normalized to the baseline value and all cohorts have been shown as the mean with error bars depicting the standard deviation. (B) Maximal increase in ALT levels during the first cycle is plotted. All values have been normalized to the baseline value. All symbols represent a single patient.



(paracetamol or APAP), which also may have contributed to liver toxicity. We plotted the levels of ALT relative to baseline to determine the changes over time for the first cycle for the different cohorts (Fig. 1A). Interestingly, a peak in ALT was seen at C1 day 15 with a trend towards increased liver enzyme levels at higher doses of reovirus. The same trend was confirmed by plotting the maximal increase during the first cycle of treatment (Fig. 1B). Despite the small patient numbers these data indicate a possible role for reovirus in exacerbating gemcitabine-induced liver enzyme elevations. Importantly, these figures show that the liver enzyme changes are quickly reversible and can only be detected by intensive sampling during treatment cycles.

None of the patients had evidence of cardiotoxicity. Three patients had transient troponin I increases that were not associated with cardiac enzyme elevations or cardiac symptoms or signs. In one of these patients, echocardiography showed a low ejection fraction (35–40%) after treatment, but this finding was not interpretable because no baseline echocardiography had been performed prior to the protocol amendment. This patient had esophageal cancer with extensive mediastinal disease, which has been reported to be associated with low ejection fraction (18). This patient had no clinical signs of cardiotoxicity. ECG performed before and after treatment in all patients did not show any clinically significant changes. Furthermore, after the protocol amendment echocardiography performed at baseline in 14 of 16 patients and 11 of these had follow-up scans during or after treatment and none showed changes from baseline.

Despite extensive sampling viral shedding was not detected in any of the samples taken.

Gemcitabine pharmacokinetics

Pharmacokinetic parameters of gemcitabine [total systemic clearance (CL) and apparent volume of distribution (Vss)] were not appreciably different when administered in combination with increasing doses of reovirus (Supple-

mental Table 1). Furthermore, pharmacokinetic parameters of gemcitabine (CL and Vss) administered in combination with reovirus were not appreciably different to corresponding parameters derived from previously conducted studies in patients with cancer using similar dosing regimens.

NARA response and immune monitoring.

Fourteen out of sixteen patients had evaluable samples for NARA determination. Unfortunately, samples for the first 2 patients were not available. The results per patient are given in Table 3. One of 14 patients reached the peak NARA titer at day 15 of cycle 1, 2/14 patients had their final NARA titer measurement at day 15 of cycle 1 and 11/14 patients did not reach their peak NARA titer at this point in time. The peak titer of NARA was $>1/10,000$ in 5/14 patients and did not reach $1/100,000$ in any of the patients tested (Fig. 2). Compared to the results in the monotherapy Phase I trial that reported NARA titers (10), the NARA response is attenuated both in time to induction, as well as peak levels (Table 4).

Response evaluation

Ten patients were evaluable for response as determined by the presence of measurable disease and receiving at least 2 cycles of treatment. One patient with metastatic nasopharyngeal carcinoma had a partial response according to RECIST after 4 cycles of treatment and 1 patient with breast cancer had a clinical improvement of symptoms and a minor decrease in tumor size. This patient had failed multiple lines of treatment including radiation, epirubicin, cyclophosphamide, docetaxel, trastuzumab, vinorelbine, and capecitabine. Unfortunately, no material was available to test *EGFR* and *KRAS* mutations in the patients that responded. Furthermore, six other patients had SD for 4 to 8 cycles, for a total disease control rate (CR+PR+SD) of 80%. The median duration of all patients with SD as best response according to RECIST was 72 days (range 36–112 days).

Table 3. NARA response per patient

Cohort no	Patient no.	Fold increase pre-treatment compared to C1D15	Fold increase pre-treatment compared to peak level	Last sample
1	00-0101	NA	NA	NA
	00-0102	NA	NA	NA
2	00-1001	No pre	No pre	C5D1
	00-1002	0	729	C4D1, EOS
	04-1003	3-27	729-2187	C6D1, EOS
3	04-1101	27-81	243	C2D15, EOS
	00-1102	3-9	3-9	C1D15
	00-1103	3	2187	C8D1
	00-1104	27-81	81	C2D15
4	04-1201	0-3	0-3	C1D15
	00-1202	3-27	81	C4D1, EOS
	00-1204	9-27	729-2187	C4D1, EOS
5	00-1301	243-729	243-729	C2D15
	00-1302	3-9	81-243	C3D1
	00-1304	27-81	243	C2D8

NA = not available; No pre = no pretreatment sample present.

Discussion

We present the results of a Phase I trial combining reovirus and gemcitabine. We did not formally determine the MTD of the combination but based on our 3 patient cohorts we consider 1×10^{10} TCID₅₀ of reovirus on day 1 and gemcitabine 1,000 mg/m² day 1 and day 8 of a 21-day cycle as the preliminary recommended Phase II dose. The combination of reovirus and gemcitabine did not alter gemcitabine pharmacokinetics and if reovirus was given on day 1 of each cycle, toxicity was predictable except for elevations in ALT. However, these liver enzyme changes are

mild, transient and the clinical relevance of these changes is unclear. Immune monitoring shows that gemcitabine attenuates the NARA response compared to previous single agent Phase I experience. The combination of reovirus and gemcitabine shows some clinical activity in this highly pretreated patient population, however confirmatory trials need to show the additional value of reovirus when combined with gemcitabine.

The current trial did not determine MTD due to a DLT constituted by a grade 3 ALT rise in the 3×10^{10} TCD₅₀ on day 1 cohort. Phase II studies using this combination should include vigorous safety evaluations tracking liver enzyme changes. However, we feel that the transient ALT rise does not necessarily constitute a major impediment for further clinical development of this combination treatment. In addition, more precise dose finding for the tolerated dose of reovirus could be part of the ongoing effort to explore this combination.

Of note, both patients who had a grade 3 liver enzyme elevation had concomitant treatment with acetaminophen. A recently published preclinical study in mice showed evidence of drug interaction (ALT elevations) when acetaminophen was coadministered with reovirus serotype 1 Lang (19). Although REOLYSIN is derived from a different strain—reovirus serotype 3 Dearing—which is known to be less virulent and hepatotoxic than serotype 1. Oncolytics reviewed the available database of 286 patients treated with reovirus for the incidence of grade 3 or greater liver enzyme elevations. Only 9 patients (3%) had grade 3 or greater enzyme elevations and 8/9 received concomitant acetaminophen and reovirus. Therefore, Gr 3 or greater liver enzyme elevations occurred in 4% of patients that received

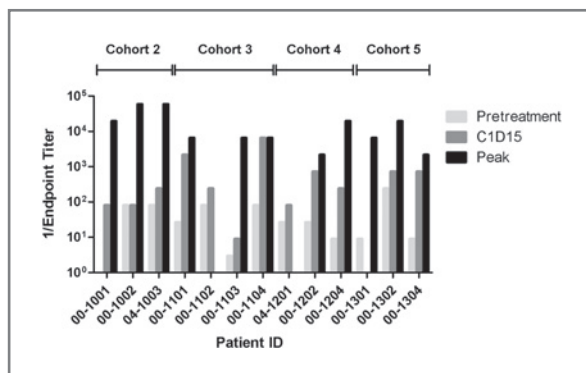


Figure 2. NARA response per patient pretreatment, C1D15 and peak neutralizing antireoviral antibody (NARA) titers for patients in the different dose levels of the trial. The y-axis represents 1/endpoint titer, meaning that the serum needed to be diluted with this factor to restore reovirus toxicity to 80% or more to L929 cells *in vitro*. Patients 00-1102 and 04-1201 had their final titer at C1D15 and patient 00-1301 did not have an evaluable C1D15 sample.

Table 4. NARA response compared between the 2 phase I studies with systemic administration reovirus

Study	Patients (N)	Treatment schedule	Peak level* ≤ day 15	Peak level ≥ 1/10,000	Peak level ≥ 1/100,000
Vidal et al./White et al.	35	Dose escalation reovirus single agent days 1–5 of 28 day cycle	20/32 (62.5%)	31/32 (96.9%)	23/32 (71.9%)
Lolkema et al.	16	Dose escalation reovirus day 1 of 21 day cycle combined with gemcitabine 1,000 mg/m ² day 1/8	1/12 (8.3%)	5/14 (35.7%)	0/14 (0%)

N.D. = not determined.

*Only patients that had their last sample beyond C1D15 were included.

concomitant reovirus and acetaminophen treatment and only in 1% who received reovirus with no recorded concomitant administration of acetaminophen. Although this difference is not statistically significant, Oncolytics concluded that there may be a theoretical risk of acetaminophen interaction with reovirus and requested that the use of acetaminophen be avoided in all reovirus trials.

Interestingly, gemcitabine affects the humoral immune response to reovirus and attenuates the NARA response. In line with our results gemcitabine has been shown in mouse models to specifically affect the generation of antibodies by B cells (20). Attenuation of a NARA response may increase exposure of normal tissues to the virus and thus increase toxicity but could also be beneficial for the delivery of virus to the tumor (8,12,11). Our study is the first to suggest that coadministration of gemcitabine potentially results in more pronounced reovirus or gemcitabine induced side effects due to attenuation of the NARA response. We are currently performing a trial to titrate cyclophosphamide doses in combination with systemic reovirus therapy. This trial has an endpoint based on NARA titers combined with toxicity and will be an important step forward in determining whether chemotherapy induced immune suppression is effective in enhancing the antitumor effect of reovirus therapy. Moreover, the combination of reovirus with gemcitabine could also have a separate effect on cellular immunity which may play a separate role in its anticancer activity (21). Gemcitabine has been reported to affect the balance of the cellular immunity towards a more effective antitumor

efficacy of the reovirus treatment independent of attenuation of the NARA response (22,23). Therefore, immune monitoring measuring both cellular components and NARA levels, should be considered as part of future trials exploring reovirus in combination with chemotherapy.

This trial had a patient with nasopharyngeal cancer that showed an objective response on imaging. This finding is potentially interesting as nasopharyngeal carcinoma is associated with infections with the Epstein Barr Virus (EBV). EBV infection is known to induce the expression of *EGFR*, a potential explanation for the observed efficacy (24). Overall these data indicate that this combination warrants further exploration.

Disclosure of Potential Conflicts of Interest

M. Coffey, K. Mettinger, and G. Gill are employed by Oncolytics Biotech and M. Coffey, K. Mettinger, and G. Gill have an ownership interest in that company; K. Harrington has received research grants from Oncolytics Biotech; J.S. De-Bono has received research support from Oncolytics Biotech

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