REO-10: A Phase 1 Study of intravenous Reovirus and Docetaxel in Patients with Advanced Cancer

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Statement of translational relevance

Oncolytic viruses are currently undergoing rapid evaluation in the metastatic cancer setting. Their true potential will be realised in combination with conventional chemotherapy, radiotherapy or targeted therapy. In particular, oncolytic reovirus has been shown in numerous single agent studies to be safe and able to target tumour sites. This study is the evolution of the original single agent systemic study (REO-005), with the addition of docetaxel to systemic reovirus. It is the first study to examine a reovirus/full dose chemotherapy combination in humans. Preclinical work has shown marked synergy both in terms of tumour kill but also mechanistically through enhancement of apoptosis and stabilisation of microtubules. In combination, reovirus was safe and no dose limiting toxicity was observed. Although the anti-tumour effects of the individual agents were not measurable, reovirus was able to track to remote tumour sites, with reoviral protein expression in metastases. This study also reiterates the feasibility of virus/chemotherapy combinations in the outpatient setting and provides the framework for phase II and phase III studies.
Abstract

Purpose: REOLYSIN® (Oncolytics Biotech) consists of a wild-type oncolytic reovirus which has selective cytotoxicity for tumour cells while sparing normal cells. In a phase I study as a single agent, repeated infusions of reovirus were safe with evidence of antitumour activity. Preclinical studies indicate potential for synergy between reovirus and chemotherapeutic agents. A multicentre, phase 1 dose escalation study was designed to assess the safety of combining reovirus with docetaxel chemotherapy in patients with advanced cancer.

Experimental Design: Patients received 75mg/m^2 of docetaxel, day 1, and escalating doses of reovirus up to 3 x 10^{10} TCID_{50}, day 1-5, every 3 weeks.

Results: 25 patients were enrolled, 24 patients exposed to treatment with 23 completing at least one cycle and 16 suitable for response assessment. Dose-limiting toxicity of grade 4 neutropaenia was seen in one patient but the maximum tolerated dose was not reached. Antitumour activity was seen with one complete response and 3 partial responses. A disease control rate (combined complete response, partial response and stable disease) of 88% was observed. Immunohistochemical analysis of reovirus protein expression was observed in post-treatment tumour biopsies from 3 patients.

Conclusion: The combination of reovirus and docetaxel is safe, with evidence of objective disease response, and warrants further evaluation in a phase II study at a recommended schedule of 75mg/m^2 of docetaxel, 3 weekly and reovirus 3 x 10^{10} TCID_{50} day 1-5, every 3 weeks.
Introduction

Reovirus type 3 Dearing (REOLYSIN; Oncolytics Biotech), Reovirus; Oncolytics Biotech), is a wild type double-stranded RNA virus which is ubiquitous and non-pathogenic in humans (1). It has been shown to be oncolytic by its ability to replicate selectively in transformed cells, but not in normal cells (2). Despite a significant humoral response, reovirus is capable of oncolysis of tumours after both local injection and systemic administration in murine models and clinical trials (3-7). Activation of the Ras pathway in transformed cells, or the upstream or downstream elements, is an important factor in a cell’s permissiveness to reovirus oncolysis (8). This is in part due to the inability of Ras-activated cells to phosphorylate cellular PKR, but also due to enhancement of virus uncoating, particle infectivity and apoptosis-dependent release (9). As mutations that activate Ras itself, or elements in its pathway, are present in over 60% of cancers (10), these cancers are therefore potential targets for reovirus oncolysis.

A number of phase 1 studies of reovirus as a single agent have been completed in patients with advanced, refractory cancer (reviewed in 11). Three early trials focused on intrallesional delivery of reovirus, which was found to be safe with evidence of response in both injected tumour and distant metastases (5). Systemic delivery of reovirus has been shown to be safe and well tolerated, with objective evidence of response in a number of cases (3,4,6,12). Notably no maximum tolerated dose has been reached in any of the trials. Recent reports by our group and others have established that the antitumour efficacy of reovirus is enhanced by combination with both
Radiotherapy or chemotherapy (7,13,14). More specifically, we and others have found reovirus in combination with taxanes results in significant synergistic tumour cell kill \textit{in vitro} and in a murine model (14). Docetaxel acts by disrupting the normal process of microtubule assembly and disassembly. Reoviruses have been shown to associate with microtubules and may require this association for efficient viral replication. We found enhanced microtubule stabilisation following combination treatment with reovirus/docetaxel (manuscript submitted). The strong synergy observed led to the selection of docetaxel for this combination study. In addition to the exploitation of oncogene signalling, reovirus activates the host immune response to potentially enhance antitumor responses through the efficient induction of type I interferons (15). Also, the local inflammatory response generated by reovirus-infected tumor cells causes bystander toxicity against reovirus-resistant tumor cells and activation of human myeloid dendritic cells (16). This phase I dose escalation study was designed to determine the maximum tolerated dose (MTD) and any dose limiting toxicities (DLT) with the combination of systemic reovirus and docetaxel. Secondary objectives included determining the effect of docetaxel on the expected humoral response to reovirus, pharmacokinetics of docetaxel when administered with reovirus, and assessment of any antitumour activity.
Materials and Methods

Patients

Patients diagnosed with advanced or metastatic solid tumours refractory to standard of care treatment, or for which no curative standard therapy existed, and for whom docetaxel was an appropriate palliative chemotherapy, were considered for enrolment. To be eligible, patients were required to have measurable or evaluable disease; no continuing residual toxic effects related to any prior anti-cancer therapy, with any such effect having resolved to Grade 1 or lower; be ≥18 years of age; have received no chemotherapy, radiotherapy, biological therapy and hormone therapy (apart from patients with breast cancer and LHRH analogues in prostate cancer) within 28 days prior to receiving the study drug; have an ECOG Performance score of ≤2 and a life expectancy of at least 3 months.

The following baseline laboratory results were required: absolute neutrophil count ≥1,500/μl, platelets ≥100,000/μl, haemoglobin ≥9.0mg/dL, serum creatinine ≤1.5 x institutional upper limit of normal, total bilirubin ≤1.5 x institutional upper limit of normal, aspartate transaminase / alanine transaminase ≤2.5 x institutional upper limit of normal; and a negative pregnancy test for females of childbearing potential. Exclusion criteria included known brain metastases, concurrent immunosuppressive therapy, known HIV, hepatitis B or C infections, pregnancy or breast-feeding, clinically significant cardiac disease (New York Heart Association class III or IV), and
dementia or altered mental state that would prohibit informed consent. The study was approved by the local ethics committees.

**Study Design**

This was an open-label, dose-escalating, non-randomised, three-centre Phase I study of reovirus given intravenously combined with docetaxel every 3 weeks. Docetaxel, following oral premedication with dexamethasone (8mg daily for the three days up to treatment), was given as a 60 minute iv infusion on day 1, every 21 days. The reovirus used in the study was an isolate of the Type 3 replication competent Dearing strain (REOLYSIN) supplied by Oncolytics Biotech Inc., Calgary, AB, Canada. Reovirus was administered to the patients as an iv infusion over 60 minutes from day 1 to day 5, every 21 days. On day 1 of each cycle, when both agents were to be given, the docetaxel was given first. Three patients were enrolled in each cohort, at the dose level shown in table 1, in order to determine the maximum tolerated dose. At the beginning of each new dose level only one patient was treated. The second and third patients of the cohort were not treated until at least 2 weeks after the initial patient in that cohort had received the first dose of reovirus.

Patients continued to receive treatment under this protocol for a maximum of eight cycles, provided there was no evidence of disease progression and the treatment was tolerated.
Dose Escalation

Patients were initially enrolled in groups of three and individually assessed for safety and dose limiting toxicities (DLT). Patients were considered evaluable for dose escalation decisions if they had received at least one cycle or withdrew from the study due to drug-related toxicity. If a patient withdrew from the study without meeting these criteria they were replaced in the cohort.

If one out of three patients in a dose group experienced a DLT during the first cycle, three more patients were added to that dose group. If two or more patients in a dose group experience a DLT during any cycle, the previous lower dose would be defined as the MTD. Intra-patient dose escalations were not permitted. Treatment was continued for a maximum of eight cycles, provided it was well tolerated and there was no evidence of disease progression.

Viral administration

Reovirus was supplied by Oncolytics Biotech in single-use 1mL glass vials containing a frozen viral suspension in PBS. Stock was stored at -70°C and thawed rapidly over 2 min, and the appropriate TCID50 dose was diluted to 250mL in 0.9% sodium chloride and infused over 1 hour through a peripheral line. Treatment was given in a side-room and patients were monitored closely (including blood pressure, temperature, and heart rate measurements) during, and for at least one hour after infusion.
Dose Limiting Toxicity

Toxicities were graded according to Common Terminology Criteria for Adverse Events version 3.0. DLT was defined as any of the following events that were determined to be possibly or probably related to combination therapy during the first cycle of treatment irrespective of whether the toxicity had resolved: absolute neutrophil count <0.5 x 10^9 lasting for more than 7 days or with sepsis, platelet count <25 x 10^9/L, grade 2 neurotoxicity or cardiotoxicity, any other drug-related non-haematological grade 3/4 toxicity, with the exceptions of flu-like symptoms, nausea and vomiting if appropriate prophylactic or therapeutic measures had not been administered, and the inability to tolerate one course of therapy due to toxicity.

In order to define DLT, patients were not given prophylactic growth factor support, antidiarrhoeals, or antipyretics during the first cycle of therapy.

Safety Evaluations

Safety was assessed by the evaluation of the type, frequency, and severity of adverse events, changes in clinical laboratory tests (including haematological, clinical chemistry and urinalysis), immunogenicity and physical examination. ECG was performed at baseline, after each reovirus infusion for the first cycle, on day 1 of each subsequent cycle and at the end of the study. Patients experiencing any DLT in any cycle had their treatment held until toxicity resolved to baseline or grade 1. Upon resolution, reovirus and docetaxel therapy was recommenced at a lower dose level.
Response Evaluation

Response was assessed by RECIST criteria (17). All patients were clinically evaluated after each course of treatment and radiologically every second course until there was evidence of progressive disease. Tumour markers were also used to assess response in appropriate patients.

Pharmacokinetic and Pharmacodynamic endpoints

To assess docetaxel pharmacokinetics blood samples were taken during the first cycle of treatment at the following time points: baseline, 15 minutes, 30 minutes, 1 hour, 2h, 4h, 6h, 8h, 24h, and 48h. Three patients whose tumours were accessible underwent biopsy to evaluate viral replication. Samples were stored at -80°C until the time of analysis. At this time, the biopsies were thawed and macerated in 1 mL DMEM and centrifuged at 3,600 rpm for 5 minutes. The supernatant was taken, serially diluted (1:10), and placed on to L929 cells in quadruplicates in a 96-well plate. The viral titre was calculated using the Kärber statistical method for a standard TCID$_{50}$ assay.

Analysis of Viral Shedding by reverse transcription-PCR

Initial evaluation of the detection limit of reovirus RNA by 35 cycles of reverse transcription-PCR (RT-PCR) was done. Viral RNA was extracted from 140μL stock using the QIAamp viral RNA mini-kit (Qiagen) and serially diluted. 5μL was assayed directly by RT-PCR using the One-Step RT-PCR Enzyme Mix Kit (Qiagen). Reovirus s3 cDNA targeted primers used were forward 5´-GGGCTGCACATTACCACTGA and reverse 5´-
CTCCTCGCAATACAACTCGT. PCR conditions were 50°C for 30 min, for reverse transcription; 95°C for 15 min; and 35 cycles of 95°C for 30 seconds, 62°C for 45 s, and 72°C for 45 s followed by 72°C for 7 min.

All patients had blood samples collected for the detection of reovirus titres during the first 2 cycles. Samples were taken at baseline, 4 hours after the last dose of reovirus on day 5 and day 15. Blood from the contra-lateral arm was collected into EDTA tubes, centrifuged at 1,200 x g for 10 min at 4°C, and stored at -70°C. Urine, sputum, and faecal swab (after PBS elution) samples were also stored at -70°C. Samples were analyzed after the last treatment dose in each cycle and weekly using the 35-cycle RT-PCR. Reovirus RNA (300-bp PCR product) and water were included in all experiments as positive and negative controls, respectively.

Detection of neutralising antireoviral antibodies

A modified neutralising antibody assay was used to detect antibody titres at baseline and weekly during the first 2 cycles of treatment by measuring the effect of patient serum samples on the ability of reovirus to kill a monolayer of target mouse L929 cells. The neutralising antireoviral antibodies (NARA) titre of serum samples was expressed as the last dilution causing <80% cell killing as described previously (18). Assays were performed in duplicate by 2 different technicians to verify the results.

Immunohistochemistry of biopsies for reoviral protein.
Where possible, fine needle core biopsies were taken from accessible metastatic sites after reovirus and docetaxel treatment, on day 5 of cycle 2. Biopsies were fixed in formalin and paraffin embedded. Immunohistochemical analysis of reovirus protein expression in 5 micron sections followed a published protocol. The Benchmark LT automated system (Ventana Medical Systems, Tucson, AZ) was used. In brief, optimal conditions were determined from blinded analysis of cells either infected with reovirus or not infected using 1:3000 diluted primary antibody following pretreatment in Ventana’s cell conditioning 1 for 30 minutes (antigen retrieval). The primary antibody was a kind gift from Dr. Matt Coffey and was derived in goat; a rabbit antigoat (ABCAM, Massachusetts) secondary antibody was used at a dilution of 1:3000. The antigen was detected with the Ultraview Universal DAB or Fast Red system from Ventana with a counterstain of hematoxylin. The negative controls included omission of the primary antibody and carcinomas from patients who had not been treated with the reovirus.

Analysis of the colocalization of microtubular protein and reovirus protein was done using the nuance system from Cambridge Research Institute. Optimal conditions for microtubular protein involved protease digestion (ventana protease 1, 4 minutes), and a dilution of 1:5. After immunohistochemical analysis of microtubular protein using dab as the chromogen, the same slide was then tested for reovirus protein using immunohistochemistry and the fast red chromogen.
Results

Patients

A total of 25 patients were enrolled into the study across three centres between June 2007 and January 2009. Their demographics are summarised in table 2. One patient failed to start treatment due to worsening liver function tests after enrolment. Another patient developed leucopenia on day 2, cycle 1 and was taken off study and replaced in the cohort. All others received at least one cycle. They were treated over three dose levels and received a total of 98 cycles (median 3; range 1-8), summarised in table 3a. Sixteen patients completed at least two cycles of treatment and were therefore eligible for response assessment.

Safety and Toxicities

The treatment was well tolerated with the most common side-effects being flu-like symptoms (fever, chills, and headache), diarrhoea, fatigue and neutropenia. The grade 3-4 toxicities are given in table 3. There were a total of six grade 4 toxicities. This included four episodes of grade 4 neutropenia in cohort 3 all of which were thought to be due to the docetaxel therapy alone. One man with prostate carcinoma and a retrovesical fistula had grade 4...
neutropaenia after his first cycle of treatment. He continued on the study on the cohort 2 dose of reovirus and a 20% reduction in docetaxel for a further 4 cycles without any further DLTs. One episode of grade 4 neutropaenia was complicated by sepsis though the patient fully recovered and completed eight cycles of treatment and the same patient developed grade 4 lymphopaenia during treatment. All other toxicities were grade 3 or less. One patient with hepatocellular carcinoma developed a grade three rise in AST on day 5 of cycle 1 and treatment was withheld. Consequently, this patient was replaced in the cohort.

Flu-like symptoms typically occurred 2-4 days after reovirus administration and were easily controlled with paracetamol and non-steroidal anti-inflammatory medication. Symptoms appeared to be more common in the first cycle of treatment and milder in subsequent cycles. The incidence of neutropaenia was not affected by the dose level of reovirus, however there was a relationship between neutropaenia and the amount of previous chemotherapy received, with all the patients who had grade 4 neutropaenia having received at least 6 cycles of chemotherapy before enrolment.

**Viral Biodistribution**

Pre-treatment and post-treatment serum, urine, saliva and anal swabs were negative in all but two patients using RT-PCR screening based on 35 cycle amplification. In patient 0308, positive signal was detected in the serum on day 5 of cycle 1. Patient 0317 had positive signal in the urine and saliva on...
day 15 of cycle 1, and also in the serum on day 5, and the anal swab on day
15 of cycle 2. This patient also had a positive reading in the pre-treatment
serum sample which may indicate contamination. The analysis of viral
shedding including representative gels is illustrated in supplementary
materials.

**NARA Response**

Twenty-two patients had data available for analysis of the increase in
neutralising antibody titres (NARA). This was expressed as the fold increase
in antibody titre compared to pre-treatment. All patients showed an increase in
NARA titres with a range of 27-2187 (median 243) at day 15, cycle 1, and 27-
6561 (median 729) at peak (Figure 1). From previous studies, evidence of
effective immune modulation was taken if the day 15, cycle 1 NARA showed
only a 10-50 fold increase over the pre-treatment control. In this study, only
one patient, 101, had evidence of immune modulation. However, this patient
had a dramatic fall in his neutrophil and lymphocyte count after the second
day of treatment and was consequently taken off study. All the other patients
had increases in their NARA of 80-fold or more, indicating that docetaxel had
no effect on the production of neutralising antibodies to reovirus.

**Response Assessment**

Sixteen patients were eligible for response assessment having completed 2
cycles of treatment (Table 4). Objective radiological responses were seen in 4
patients. There was one complete response in the liver of a patient with
metastatic breast carcinoma. This patient completed eight cycles of treatment with no evidence of disease recurrence in the liver at the end of study. Three patients had objective evidence of a partial response: a patient with ocular melanoma had a 30% reduction in the size of liver metastases, a patient with gastric carcinoma had a 32% reduction in the size of target lymph node metastases, and a patient with gastro-oesophageal carcinoma had a 32% reduction in lung metastases. Ten patients had evidence of stable disease for at least 2 cycles, six of whom completed at least six cycles of treatment. Three patients with radiologically stable disease had evidence of a minor response: a patient with mesothelioma, who received six cycles, had a 23% decrease in the size of a target mediastinal lymph node, a patient with a pancreatic carcinoma had a 48% decrease in Ca19.9 tumour marker, and there was a 30% fall in PSA in a patient with prostate carcinoma.

**Post treatment biopsy evaluation**

Where possible, fine needle core biopsies of accessible metastases were taken on day 5, cycle 2 of treatment. Evaluable tissue was obtained from three patients with: prostate cancer (iliac lymph node biopsy), unknown primary tumour (liver biopsy) and pleural mesothelioma (pleural biopsy) and immunohistochemistry for reovirus protein completed. Figure 2A-E. Compared to control sections of normal human liver, reoviral protein expression was observed in tumour cells in all 3 biopsies. The staining was mainly cytoplasmic and the strongest expression was seen in metastatic mesothelioma.
In one patient we were able to demonstrate co-localisation of reovirus to microtubule protein consistent with virus replication in the tumour cells, figure 2 F-I.

**Pharmacokinetics**

The effect of reovirus on docetaxel pharmacokinetics was assessed by the measurement of serial serum samples after intravenous delivery of docetaxel. All patients had similar results with no difference in docetaxel clearance than would be expected in patients receiving docetaxel alone. Peak concentration of docetaxel ranged from 1510-4080 ng/ml between 15 minutes and 30 minutes after docetaxel administration. The concentration of docetaxel fell below 100ng/ml within two hours in all patients. There was no correlation between docetaxel clearance and dose escalation of reovirus.
Discussion

The purpose of this study was to determine the safety, DLT and MTD of intravenous reovirus in combination with docetaxel chemotherapy in patients with advanced solid malignancies. In keeping with experience with other viral therapies, it was always anticipated that the therapeutic potential of reovirus would be realised through combination with other anticancer agents. Supporting preclinical data suggested strong potential synergy between the two agents through enhanced tumour apoptosis. This study also allowed an evaluation of any immunomodulatory effects of docetaxel on the humoral response to reovirus and also whether concurrent treatment with reovirus affected the pharmacokinetics and clearance of docetaxel.

A number of chemotherapy/oncolytic virus combinations have been evaluated to date, and have been shown to result in marked antitumor effects without compromising safety. Onyx-015, an oncolytic adenovirus engineered to replicate in p53 mutant tumour cells, showed enhanced clinical efficacy when
combined intratumorally with systemic cisplatin and 5-fluorouracil, compared to chemotherapy alone (19). A large number of pre-clinical reports indicate marked synergy between oncolytic viruses, with varied mechanisms of action, and a range of chemotherapeutic agents (20-25). Not surprisingly, the mechanisms underlying the observed synergies are incompletely understood.

As a single agent, reovirus has been shown to be safe in human use, and has been associated with minimal toxicity when given intravenously or intratumorally in patients with a wide range of solid cancers (4,6). Anti-tumour effects have been regularly observed even in phase I studies, (reviewed in 26)

The toxicities seen in this study included fever, flu-like symptoms, fatigue and nausea, and were similar to what has been reported with either agent alone. As only one DLT, of grade 4 neutropaenia, was encountered the MTD was not technically reached. However, the highest dose of reovirus available for administration is $3 \times 10^{10} \text{TCID}_{50}$ and this combined with docetaxel $75 \text{mg/m}^2$ is the recommended dose for ongoing studies.

As in previous trials of single agent reovirus, viral shedding was observed infrequently (two patients). One had positive samples from the serum, saliva, urine and anal swab, while the other patient just had a positive serum sample. The results suggest that there is rapid clearance of the virus from the circulation which is unaffected by the administration of docetaxel. There was a rapid induction of a humoral response to reovirus with all patients showing an increase in the NARA titres after the first cycle. Despite its reported
immunomodulatory effects (27), docetaxel appeared to have no effect on the NARA response. From the single agent reovirus study by Vidal et al the median increase in NARA titres above baseline was 250-fold. They proposed that successful immune modulation could be defined as a rise in NARA titre that is at least 10-fold lower than observed in the single agent reovirus studies. Only one patient in this study met that criteria and he had an unusual and dramatic fall in his neutrophil and lymphocyte count on cycle 1, day 2 and was taken off study. It is possible that the reduced exposure to reovirus and myelosuppression may have limited the extent of the NARA response. The potential reduction/elimination of reovirus oncolysis due to the NARA response has been extensively debated. In our view it is most likely that NARA has a role in preventing the efficient spread of progeny but not viraemia. The virus may gain access to immune privileged areas of tumours and escape detection. There is additional evidence that reovirus persists for several months post treatment despite NARA and late tumour marker and radiological antitumour responses have been observed. (4-6)

The clearance of docetaxel was similar in all patients and across all cohorts with no obvious relationship to the reoviral dosage.

While not a primary endpoint of the study, objective radiological tumour responses were seen. Given that all patients were docetaxel-naïve it is impossible to determine whether responses were a result of either agent alone, or their combination. Only two patients had not received prior chemotherapy, both had hormone refractory prostate cancer. The majority of
patients had received one previous line of chemotherapy, though six patients had two or more lines of chemotherapy. Of note two patients had received prior paclitaxel chemotherapy. One of these with breast cancer and liver metastases completed 8 cycles of treatment on the study with a complete response in her liver. The other patient had carcinoma of unknown primary site and completed six cycles of treatment with stable disease.

Partial responses were seen in three further patients with ocular melanoma, gastric carcinoma, and oesophageal cancer. All three completed at least six cycles of treatment. A further ten patients had stable disease as a best response and only two of the evaluable patients had disease progression after the first two cycles. Taken together this translates into a disease control rate of 88% and an objective response rate of 25%. While this response rate fits in with reported response rates for second line single agent docetaxel (melanoma 14% (28); gastric 20% (29); breast 30-50% (30), the high disease control rate and response in two patients previously treated with a taxane is encouraging. In our previous study REO-005 where reovirus was administered as a single agent, we were able to isolate and propagate reovirus from post-treatment tumour biopsy tissue. We were unable to demonstrate this in the current studies, however, in all three patients where post treatment tumour biopsies were taken, we found reoviral protein expression in tumour cells by immunohistochemistry, – which is clear evidence of virus tracking to sites of metastases. The reovirus staining was mainly cytoplasmic and largely restricted to tumour cells with much lower expression in normal adjacent tissue (liver and fibroblasts). Furthermore, in
one biopsy, we demonstrated co-localisation of replicating virus in microtubular protein consistent with proliferating virus in the tumour.

Reovirus as a single agent is safe and demonstrates modest efficacy. However, the future place of reovirus as an anticancer agent is likely to depend on strategies that improve systemic delivery to the tumour, avoid rapid viral clearance by the immune system, and combine reovirus with other anticancer agents. Here we present the first clinical trial of reovirus combined with a chemotherapeutic agent. The combination is safe and the recommended dose for future studies is $3 \times 10^{10}$ TCID$_{50}$ of reovirus and 75mg/m$^2$ of docetaxel. Disease stabilisation rates for this combination are promising and further studies are warranted.
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References


Figure Legends

Figure 1. Fold increase in reoviral antibody titre.
Graph shows the fold increase in reoviral antibody titre over pre-treatment in the 22 patients for whom data were available. Both the cycle 1, day 15 (white) and peak (grey) reoviral antibody titre are shown.

Figure 2 A-E. Expression of reoviral protein (red stain) in post treatment biopsies. A: normal liver X400; B: liver biopsy from metastasis from carcinoma unknown primary x400; C: normal liver adjacent to liver metastasis from carcinoma; D: metastatic prostate cancer in an iliac lymph node X200; E: Pleural biopsy malignant mesothelioma X400. Large arrow indicates reoviral protein in tumour cells (red) with no staining in supporting fibroblasts, thin arrow.

Figure 2 F-I. RGB image analysis of tissue with reovirus (red) and microtubular protein (brown) using the nuance system. This system converts the RGB image of the DAB signal to fluorescent green (microtubular protein, panel G), the RGB image of the fast red system to fluorescent red (reovirus, panel H), then mixes them with the fluorescent green representing cells that express the reovirus in the microtubular complex (I) arrow. X400
Table 1 - Dose level by cohort

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- Necessary only if toxicity is encountered at the initial dose level.
Table 2 – Patient Characteristics

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<td>0</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3a Patients treated at each dose level and Grade 4 events observed

<table>
<thead>
<tr>
<th>Cohort</th>
<th>No. Of Patients</th>
<th>Reovirus Dose (TCID&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>Total Number of Cycles (Range)</th>
<th>Grade 4 Event (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>$3 \times 10^9$</td>
<td>16 (1-6)</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>$1 \times 10^4$</td>
<td>20 (2-8)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>$3 \times 10^4$</td>
<td>62 (1-8)</td>
<td>4 *</td>
</tr>
</tbody>
</table>

*Grade 4 neutropenia (one of them developed Grade 4 lymphopenia)
Table 3b Grade >3 toxicity observed for each reovirus dose level (n=24)

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Dose Level</th>
<th></th>
<th></th>
<th>Total N=24 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>6 (25)</td>
</tr>
<tr>
<td>Neutropenia with fever</td>
<td></td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1</td>
<td></td>
<td></td>
<td>2 (8)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Bowel obstruction</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Nausea</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Dehydration</td>
<td>2</td>
<td></td>
<td></td>
<td>2 (8)</td>
</tr>
<tr>
<td>Gastric Bleed</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Abnormal ALP</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Perianal abscess</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Hypokalaemia</td>
<td>1</td>
<td></td>
<td></td>
<td>1 (4)</td>
</tr>
</tbody>
</table>
Table 4 – Antitumour activity in Evaluable Patients (n=16)

<table>
<thead>
<tr>
<th>Best Response</th>
<th>No. of Patients</th>
<th>Tumour Types</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial Response</td>
<td>4</td>
<td>Breast, Stomach, Gastro-oesophageal, Ocular Melanoma</td>
<td>CR in liver; SD in bone ↓32% in lymph nodes</td>
</tr>
<tr>
<td>Minor response</td>
<td>3</td>
<td>Mesothelioma, Prostate, SCC H + N</td>
<td>↓23% in lymph nodes ↓30% in PSA ↓26% in lymph node</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>7</td>
<td>Prostate, Unknown 1º Melanoma, Oesophagus Pancreas</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1
REO-10: A Phase 1 Study of intravenous Reovirus and Docetaxel in Patients with Advanced Cancer

Charles Comins, James Spicer, Andrew Protheroe, et al.

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